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<b>(21) International Application Number:</b> PCT/US97/05556 <b>(22) International Filing Date:</b> 4 April 1997 (04.04.97)  <b>(30) Priority Data:</b> 08/682,433 17 July 1996 (17.07.96) US  <b>(71) Applicant:</b> MEDTRONIC, INC. [US/US]; 7000 Central Avenue N.E., Minneapolis, MN 55432 (US).  <b>(72) Inventors:</b> STOKES, Kenneth, B.; 17581 Eidelweiss Street N.W., Anoka, MN 55304 (US). MORISSETTE, Josée; Apartment 306, 1101 Paul Parkway, Blaine, MN 55434 (US).  <b>(74) Agents:</b> PRESTON, Albert, W., Jr. et al.; Woodcock, Washburn, Kurtz, Mackiewicz & Norris LLP, 46th floor, One Liberty Place, Philadelphia, PA 19103 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> SYSTEM FOR ENHANCING CARDIAC SIGNAL SENSING BY CARDIAC PACEMAKERS THROUGH GENETIC TREATMENT  <b>(57) Abstract</b>  The present invention provides delivery systems for delivering ion channel protein genetic material to cardiac cells in areas adjacent to where an electrode is to be positioned in a patient's heart to improve or correct the signal to noise ratio of cardiac signals, such as the P-wave. More specifically, there is provided a system for delivering sodium ion channel proteins or nucleic acid molecules encoding sodium ion channel proteins to a site in the heart adjacent to an electrode to increase the expression of the same, thereby enhancing the cardiac signal amplitude and enabling improved sensing of cardiac signals by an implanted pacemaker.		

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SYSTEM FOR ENHANCING CARDIAC SIGNAL SENSING  
BY CARDIAC PACEMAKERS THROUGH GENETIC TREATMENT

FIELD OF THE INVENTION

The present invention relates to systems for genetically enhancing cardiac signals for use by cardiac pacemakers and, more particularly, for enhancing the signal to noise ratio of atrial P-waves for improved pacemaker sensing.

BACKGROUND OF THE INVENTION

The cardiac pacemaker is a widely used device for treating various cardiac disorders, e.g., sick sinus syndrome, "brady-tachy syndrome" and heart block. The basic function of the pacemaker is to deliver stimulus pulses to one or more of the patient's heart chambers, as and when needed, to initiate cardiac depolarizations and thus maintain a desired heart rate, or to affect improvements in cardiac output for patients in heart failure. In addition to delivering stimulus pulses, another important feature is the sensing of a patient's heartbeat signals, when they occur spontaneously, for purposes of controlling the stimulus pulse delivery. Thus, the demand pacemaker inhibits delivery of a stimulus pulse and resets the pulse generator in the event of sensing a timely spontaneous beat, i.e., a P-wave which is an atrial depolarization, or a QRS, or just R-wave, which is a ventricular depolarization. For

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example, an AAI mode pacemaker both paces and senses in just the atrium, and inhibits delivery of a pace pulse if a timely P-wave is sensed. The inhibit operation necessarily depends upon reliably sensing spontaneous P-waves. In a dual  
5 chamber pacemaker, both the P-wave and R-wave are sensed. As examples of dual chamber pacemakers, see U.S. Patents 4,920,965; 4,539,991; and 4,554,921, incorporated herein by reference. A particular purpose of the dual chamber  
10 pacemaker may be to treat a block condition, where the patient's natural pacemaker is operating normally, causing timely atrial contractions, but the depolarization signal is not efficiently propagated to the ventricle so as to cause a following ventricular contraction. In such a situation, the  
15 dual chamber pacemaker is designed to sense the P-wave, and deliver a synchronized ventricular stimulus pulse, i.e., a pulse which stimulates the ventricle after a timed AV delay which approximates the AV delay of a healthy heart. It is seen that reliable sensing of the P-wave is vital to this type of dual chamber pacing.

20 In yet another type of pacemaker operation, the pacemaker operates in what is referred to a VDD mode, meaning that it paces only in the ventricle, but senses both P-waves and R-waves, i.e., has single chamber pacing but dual chamber sensing. The advantage of this mode is that  
25 only one lead need be positioned in the patient's heart, since no pacing pulses are delivered to the atrium. The VDD lead has the normal electrode or electrode pair at its distal end, for positioning in the ventricle; and it has a "floating" electrode (or electrode pair) proximal to the tip  
30 and positioned so that it is located in the atrium, for sensing the P-wave. See, for example, U.S. Patent No. 5,127,694. However, since such a floating electrode is not necessarily embedded into or positioned adjacent the myocardium, the sensed P-wave is not as strong as for the  
35 case where a separate atrial lead is used, and consequently, the reliability of sensing the P-wave is even less.

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Atrial sensing is additionally considered to be a significant problem because of the low P-wave amplitudes commonly available and the presence of relatively large far field QRS and other "noise" signals. It is commonly  
5 accepted that atrial P-wave amplitudes are relatively low compared to ventricular R-waves because of the differences in muscle mass near the electrodes. That is, ventricular R-waves are large because there is a large volume of myocardium around the electrode, whereas the atrial signal  
10 is small because the underlying tissue is relatively thin. Thus, for any pacing system which senses the P wave, such as an AAI pacer or any dual sense mode pacer, reliably sensing P-waves is a major problem for which improvement has long been sought.

15 With regard to the source of the P-wave, it is noted that it is not the muscle itself that is sensed, but the electric potentials resulting from the depolarization of several myocardial cells, i.e., a net positive ion flow into myocardial cells through specialized membrane proteins  
20 called voltage-gated ion channels, such as the sodium channels. More muscle mass means there are more membrane channels in the area adjacent to the electrodes. However, the muscle mass adjacent to the atrial electrode cannot be increased. But the P-wave could be enhanced if the number  
25 of conducting membrane channels within the adjacent muscle mass can be increased. Sodium channels are transmembrane proteins responsible for the rapid transport of Na<sup>+</sup> ions across cell membranes underlying the depolarization of the action potential in many types of cells. In particular,  
30 cardiac fast sodium channels are responsible for the fast upstroke or phase 0 of the action potential in myocardial cells. Fozzard, et al., *Circ. Res.*, 1985, 56, 475-485. Recently, a human cardiac voltage-dependent sodium channel, hH1, has been cloned, sequenced, and functionally expressed.  
35 Gellens, et al., *Proc. Natl. Acad. Sci. USA*, 1992, 89, 554-558.

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Gene therapy has also recently emerged as a powerful approach to treating a variety of mammalian diseases. Direct transfer of genetic material into myocardial tissue *in vivo* has recently been demonstrated to  
5 be an effective method of expressing a desired protein. For example, direct myocardial transfection of plasmid DNA by direct injection into the heart of rabbits and pigs (Gal, et al., *Lab. Invest.*, 1993, 68, 18-25), as well as of rats (Acsadi, et al., *The New Biol.*, 1991, 3, 71-81), has been  
10 shown to result in expression of particular reporter gene products. In addition, direct *in vivo* gene transfer into myocardial cells has also been accomplished by directly injecting adenoviral vectors into the myocardium. French, et al., *Circulation*, 1994, 90, 2415-2424, and PCT  
15 Publication WO 94/11506.

Pursuant to the above, this invention provides a system for enhancing the cardiac pacemaker atrial and/or ventricular sensing function, i.e., enhancing the signal to noise ratio of cardiac signals, and in particular the sensed  
20 P-wave, through concurrent genetic treatment whereby the number of ion channels responsible for depolarization of the atrial or ventricular myocardial cells is increased. Applicants' invention is directed to delivery systems for introducing ion channel protein genetic material into  
25 myocardial cells adjacent to or closest to the position of the atrial or ventricular electrode. In any particular application, the genetic material is placed so as to provide maximum benefit for sensing P-waves, or other cardiac signals, with the pacing lead used, i.e., for an AAI pacing  
30 system, a lead which is fixated against the atrial wall.

#### SUMMARY OF THE INVENTION

In accordance with the above, a primary purpose of Applicants' claimed invention is to provide delivery systems for enhancing cardiac pacemaker signal sensing. In a  
35 particular embodiment, the claimed invention provides delivery systems for enhancing cardiac pacemaker P-wave

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sensing. Upon identifying a patient in which the signal to noise ratio for atrial or ventricular sensing is problematic, ion channel protein genetic material is selected such that expression of a selected ion channel protein in cells adjacent to the position of the atrial or ventricle electrode corrects or improves the signal to noise ratio for cardiac signal sensing. Preferably, expression of a selected ion channel protein can improve or correct the signal to noise ratio for cardiac signal sensing in either or both the ventricles and atria of all persons with pacemakers, especially those persons which have been diagnosed with a low signal to noise ratio for P-wave sensing. Improvement or correction of P-wave sensing can be manifested by an increase in the amplitude of the P-wave, or other characteristic of the cardiac signal, thus resulting in an increase of the signal to noise ratio of the signal sensed in the pacemaker atrial sensing channel. Delivery of the ion channel protein genetic material can be accomplished by adaptation of available pacing leads, such as, for example, AAI or DDD leads, as well as by specific modification of leads and catheters. Delivery of the genetic material may be affected by a pump or may be passive.

The ion channel protein genetic material used in the system and method of this invention comprises recombinant nucleic acid molecules comprising a nucleic acid molecule encoding the ion channel protein inserted into a delivery vehicle, such as, for example, plasmids or adenoviral vectors, and the appropriate regulatory elements. Alternatively, the ion channel protein genetic material comprises the ion channel protein itself. Expression of the desired ion channel protein from recombinant nucleic acid molecules is controlled by promoters, preferably cardiac tissue-specific promoter-enhancers, operably linked to the nucleic acid molecule encoding the ion channel protein. The conduction protein is preferably a sodium ion channel protein, such as, for example, the voltage-dependent sodium

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channel hH1, which is used to correct or improve the signal to noise ratio of cardiac signals, and in particular, atrial P-wave sensing. The ion channel protein genetic material is delivered to specific sites adjacent to the atrial or  
5 ventricular electrode within the heart by perfusion or injection of a therapeutically effective amount, which is that amount which corrects or improves the signal to noise ratio of the cardiac signal of the myocardial cells adjacent to the electrode. The therapeutically effective amount can  
10 be delivered to the specific site in the heart in a single dose or multiple doses, as desired.

The present invention provides a delivery system for delivering a therapeutically effective amount of a predetermined ion channel protein genetic material to an  
15 identified cardiac location adjacent the atrial or ventricular electrode, the genetic material being selected for amplifying the particular cardiac signal, such as, for example, the P-wave, from cardiac cells to which it is delivered, thus improving or correcting the cardiac signal  
20 to noise ratio received by the sensing electrode. The delivery system includes the selected genetic material contained in a reservoir, and a catheter or electrode subsystem for delivering the genetic material from the reservoir to the identified cardiac location so as to  
25 contact a plurality of cells in the proximity of the sensing electrode.

The delivery system may utilize an external reservoir for providing the genetic material, or alternately may utilize an implantable reservoir. In either embodiment,  
30 a controllable pump mechanism may be provided for transferring therapeutic doses of the genetic material from the reservoir, through a catheter or electrode, and to the selected cardiac location. The pump may be a mini or micro pump located within the delivery system. Alternatively,  
35 rather than using a pump mechanism, the ion channel protein genetic material can be passively delivered to the appropriate location adjacent the appropriate electrode.



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The catheter subsystem may be of a type for direct introduction into the myocardium, as with a transthoracic procedure, or, more preferably, a endocardial catheter having a distal tip portion adapted for positioning and injecting the genetic material into the myocardium from within a heart chamber. In a preferred embodiment, the catheter distal tip has a normally withdrawn helical needle, which is extendable when positioned in the vicinity of the selected site so as to be screwed into the heart. The needle is hollow and connects with the catheter lumen so as to receive the pumped genetic material; it has one or more ports located so as to effectively release the genetic material for transduction into the cardiac area adjacent the sensing electrode. In the case of an electrode subsystem, an implantable electrode is used in place of the catheter subsystem, which is able to deliver drugs, such as steroids, or other bioactive agents, such as, for example, ion channel protein genetic material. Such implantable electrodes with drug dispensing capabilities are set forth in U.S. Patents 4,711,251, 5,458,631, 4,360,031, and 5,496,360, each of which are incorporated herein by reference. The delivery system can be used for one treatment and then removed, or can be implanted for subsequent treatments, in which latter case it is controllable by an external programmer type device. In another embodiment, the catheter or electrode subsystem may be combined with a pacing lead for sensing the patient's cardiac signals and for providing stimulus pulses.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a flow diagram presenting the primary steps involved in the practice of this invention, including selecting an appropriate genetic material, positioning delivery system against the heart wall, and expressing the genetic material in an appropriate dose into the determined location.

Figure 2 is a schematic representation of a delivery system in accordance with this invention,

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illustrating delivery of genetic material into a patient's heart at the chosen location using a catheter subsystem.

Figure 3 is a schematic drawing of the distal portion of a catheter which can be used for injecting a solution carrying chosen genetic material into a patient's heart.

Figure 4 illustrates the distal end of a catheter, having a distal portion which encloses an osmotic pump.

Figure 5A is a schematic representation of a delivery system in accordance with this invention, having a combined catheter and pacing lead, with a separate pump; Figure 5B is another embodiment of a combined pacing lead and delivery catheter having a reservoir located at the distal end of the catheter.

#### 15 DESCRIPTION OF THE PREFERRED EMBODIMENTS

Applicants' invention provides delivery systems for correcting or improving cardiac signal sensing, especially the signal to noise ratio of the atrial P-wave, thus enhancing pacemaker sensing. A problematic signal to noise ratio for P-waves results from a naturally low amplitude P-wave generated in the atrium, noise from the ventricular QRS complex, muscle noise, noise from other sources, or a combination thereof. The signal to noise ratio is determined by routine and conventional techniques known to the skilled artisan. Once the specific problem has been identified in a particular patient, e.g., in any patient with a pacemaker or who is to receive a pacemaker, ion channel protein genetic material is selected such that expression of a selected ion channel protein corrects or improves the cardiac signal amplitude, thus improving or correcting the cardiac signal to noise ratio. The ion channel protein genetic material comprises either the ion channel protein itself or recombinant nucleic acid molecules comprising a nucleic acid molecule encoding the ion channel protein inserted into a delivery vehicle, such as, for example, plasmid, cosmid, YAC vector, viral vectors, and the

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like, and the appropriate regulatory elements. In preferred embodiments of the present invention, the nucleic acid molecule encoding the ion channel protein is the full length coding sequence cDNA of an ion channel protein, and is  
5 inserted into a plasmid or adenoviral vector, such as, for example, pGEM3 or pBR322, and Ad5, respectively. The regulatory elements are capable of directing expression in mammalian cells, specifically human cells. The regulatory elements include a promoter and a polyadenylation signal.

10 Expression of the desired ion channel protein is preferably controlled by cardiac tissue-specific promoter-enhancers, operably linked to the nucleic acid molecule encoding the ion channel protein. The ion channel protein is preferably a sodium channel protein, such as, for example, the hH1  
15 voltage-regulated sodium channel, which is used to correct or improve the cardiac signal to noise ratio. The ion channel protein genetic material is preferably delivered in a pharmaceutical composition comprising, for example, the ion channel protein genetic material in a volume of  
20 phosphate-buffered saline with 5% sucrose. In some embodiments, the ion channel protein genetic material is delivered with genetic material encoding the Na<sup>+</sup>/K<sup>+</sup> pump, which is also inserted into an appropriate delivery vehicle. The ion channel protein genetic material may also be  
25 delivered separately or in combination with class I and class IV antiarrhythmic drugs, which have been shown to increase sodium channel mRNA expression. The ion channel protein genetic material is delivered to specific sites within the heart, adjacent to the atrial or ventricular  
30 electrode, by perfusion or injection of a therapeutically effective amount, which is that amount which corrects or improves the cardiac signal to noise ratio. Preferably, the therapeutically effective amount corrects or improves the P-wave signal to noise ratio. The therapeutically effective  
35 amount can be delivered to the specific site in the heart in single or multiple doses, as desired, using the delivery systems of the invention.

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The present invention comprises a delivery system for delivering a therapeutically effective amount of ion channel protein genetic material to a specific cardiac location, adjacent the atrial or ventricular electrode, in  
5 such a way as to enhance the amplitude of the cardiac signal, thus improving or correcting the signal to noise ratio. In a first embodiment, the delivery system basically comprises a reservoir subsystem for holding the genetic material, and a catheter subsystem in communication with the  
10 reservoir subsystem for placement of the genetic material in and around the identified cardiac location. In another embodiment, the delivery system basically comprises a reservoir subsystem for holding the genetic material, and a  
15 electrode subsystem in communication with the reservoir subsystem for placement of the genetic material in and around the identified cardiac location. As seen in the following discussion of several preferred embodiments, the reservoir subsystem and catheter subsystem or electrode subsystem may be separate, or they may be combined.  
20 Preferably the reservoir contains up to 25 ml of a genetic material for delivery to the myocardium. In some applications, only a bolus of about 0.1-10 ml, or more preferably 1-5 ml, is delivered to the targeted areas. In other applications, such as where ion channel protein is  
25 being delivered in repeated doses, 25 ml or more may be used. Also, the genetic material may be diluted in a saline solution, such as, for example, phosphate-buffered saline (PBS), the reservoir holding the diluted solution for controlled delivery. Additionally, it is to be understood  
30 that the reservoir and associated control apparatus may be either implantable or external to the body, depending upon the circumstances, e.g., whether metered doses are to be administered to the patient over a period of time, or whether the delivery of the genetic material is essentially  
35 a one time treatment.

Referring now to Fig. 1, the primary steps involved in the practice of this invention are shown in the

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flow diagram. The illustrated steps are performed following the initial diagnosis of a patient with a problematic P-wave signal to noise ratio, which can result from a low amplitude P-wave generated in the atrium, noise from the ventricular

5 QRS complex, noise from other sources, or a combination thereof. Diagnosis can be accomplished, for example, by electrocardiography procedures. Preferably, the steps are performed in connection with all patients having cardiac pacemakers. As illustrated in block 30, the next step is to

10 select the appropriate ion channel protein genetic material. This selection yields the "preselected genetic material." The ion channel protein genetic material is next prepared, as illustrated in block 31, by either inserting the nucleic acid molecules encoding the appropriate ion channel protein

15 into a delivery vehicle with the appropriate regulatory elements, in the case of a recombinant nucleic acid molecule, or expressing the ion channel protein from an expression vector, in the case of the ion channel protein itself. As shown in block 32, the next step is to prepare

20 and load the delivery system with a therapeutically effective amount of the ion channel protein genetic material. As illustrated in block 33, the next step comprises inserting the catheter, or other delivery subsystem, such as, for example, the electrode subsystem,

25 into the patient's heart and positioning it against the heart wall. As shown in block 34, the next step comprises administering the therapeutically effective amount to the patient by contacting the appropriate location in the heart, adjacent to the atrial or ventricular electrode, using the

30 delivery system described herein. An alternative method of administering the therapeutically effective amount of the ion channel protein genetic material is to directly inject the heart of the patient. The next step, shown in block 35,

35 is to pace the patient in a standard manner, e.g., dual chamber synchronous pacing which includes sensing the patient's P-waves and delivering synchronized ventricular stimulus pulses, or AAI pacing. In accordance with this

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step, it may be preferable to adjust the sensitivity of the atrial or ventricular sensing channel in accordance with the observed cardiac signal amplitude. The final step 36, which is optional, is to evaluate the response of the patient to the treatment by, for example, measuring the amplitude of the cardiac signal, such as, for example, the P-wave, by conventional electrocardiographic techniques, such as, for example, by telemetry from the implanted pulse generator. The sensitivity can then be adjusted accordingly.

10 Referring now to Fig. 2, there is shown an illustrative embodiment of a delivery system useful for certain applications of this invention, e.g., where larger amounts of genetic material alone or in solution are employed. A catheter 38, preferably a transvenous catheter, 15 includes an elongated catheter body 40, suitably an insulative outer sheath which may be made of polyurethane, Teflon, silicone, or any other acceptable biocompatible plastic. The catheter has a standard lumen (illustrated in Fig. 3) extending therethrough for the length thereof, which 20 communicates through to a hollow helical needle element 44, which is adapted for screwing into the patient's myocardium. The outer distal end of helical element 44 is open or porous, thus permitting genetic material in fluid form to be dispensed out of the end, as is discussed in more detail 25 below in connection with Fig. 3. At the proximal end of the catheter, a fitting 46 is located, to which a Luer lock 48 is coupled. Luer lock 48 is coupled to the proximal end of sheath 40 and receives the lumen. A swivel mount 50 is mounted to Luer lock 48, allowing rotation of the catheter 30 relative to Luer lock 52. Luer lock 52 in turn is coupled through control element 54 to a tube 58 which communicates with reservoir 55, suitably through flow control 57 and filter 56. Reservoir 55 holds a supply of the selected genetic material. Control elements 57 and 54 are used for 35 adjustment of the pressure and flow rate, and may be mechanically or electronically controlled. Thus, unit 54 or 57 may be used to control either rate of delivery, or dosage

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size, or both. Control unit 54 may be programmed to automatically release predetermined doses on a timed basis. Further, for an implanted system, control unit 54 may be activated from an external programmer as illustrated at 53.

5 Reference is made to international application published under the PCT, International Publication No. WO 95/05781, incorporated herein by reference, for a more detailed description of such a reservoir and catheter combination. It is to be understood that such a system is useful for this  
10 invention primarily for applications where larger fluid amounts are to be expressed, e.g., where a diluted saline solution is used to wash or perfuse a selected area.

Referring now to Fig. 3, there is shown in expanded detail a schematic of the distal end of the  
15 catheter of Fig. 2, illustrating the interconnection of the helical element 44 with the interior of the catheter. As illustrated, the helical needle 44 is provided with an internal lumen 59 which is in communication with the internal lumen 63L of the lead formed by tube 63. In this  
20 embodiment, helical element 44 may also be a pacing electrode, in which case it is formed of conductive material and welded, or otherwise fastened, to tip element 61. Tip element 61 in turn is electrically connected to coil or coils 64, 65, which extend the length of the lead and are  
25 connected to a pacemaker. An outer membrane 60 forms the outer wall of elongated catheter body 40, shown in Fig. 2. Further referring to Fig. 3, element 44 has an outlet 75 where the genetic material may be expressed, and holes or ports 76, 77, and 78 may also be utilized for providing  
30 exits for the genetic material which is supplied through lumen 59 under a suitable pressure of zero up to about one atmosphere from reservoir 55 (shown in Fig. 2) and the control elements.

In practice, a catheter 38 of the form illustrated  
35 in Figs. 2 and 3 is advanced to the desired site for treatment, eg, adjacent the site where the sensing electrode is to be positioned. The catheter may be guided to the

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indicated location by being passed down a steerable or guidable catheter having an accommodating lumen, for example as disclosed in U.S. Patent No. 5,030,204; or by means of a fixed configuration guide catheter such as illustrated in 5 U.S. Patent No. 5,104,393. Alternately, the catheter may be advanced to the desired location within the heart by means of a deflectable stylet, as disclosed in PCT Patent Application W0 93/04724, published March 18, 1993, or by a deflectable guide wire as disclosed in U.S. Patent No. 10 5,060,660. In yet another embodiment, the helical element 44 may be ordinarily retracted within a sheath at the time of guiding the catheter into the patient's heart, and extended for screwing into the heart by use of a stylet. Such extensible helical arrangements are well known in the 15 pacing art, and are commercially available.

It is to be understood that other forms of the reservoir subsystems and catheter subsystems are within the scope of this invention. Reservoir embodiments include, for example, drug dispensing irrigatable electrodes, such as 20 those described in U.S. Patent 4,360,031; electrically controllable, non-occluding, body implanting drug delivery system, such as those described in U.S. Patent No. 5,041,107; implantable drug infusion reservoir such as those described in U.S. Patent No. 5,176,641; medication delivery 25 devices such as those described in U.S. Patent 5,443,450; infusion pumps, such as SYNCHROMED® made by Medtronic, Inc.; and osmotic pumps, such as those made by Alza.

Referring now to Fig. 4, there is shown, by way of illustration, another embodiment of a delivery system having 30 a combined catheter and reservoir, useful for applications involving delivery of a relatively small bolus of genetic material, e.g., 1-5 ml. Fig. 4 illustrates the distal end of a catheter, having a distal portion 70 which encloses an osmotic pump. See U.S. Patent 4,711,251, assigned to 35 Medtronic, Inc., incorporated herein by reference. The pump includes an inner chamber 68 and an outer chamber 66, which chambers are separated by an impermeable membrane 67. A



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semi-permeable outer membrane 72 forms the outer wall of chamber 66. The tubular portion 74 of the helical member connects to lumen 74L within inner chamber 68. A conductor 80, which runs the length of the catheter, extends into the inner chamber 68 and connects with extension 74E as shown at 74C to provide electrical contact through to element 44, in an application which the element 44 is used as a pacing electrode. A insulating cover 86 encompasses the conductor 80 from the point of contact with the semi-permeable outer membrane 72 distally. A seal 79 is provided at the point where the conductor passes through outer membrane 72 and inner membrane 67. An end cap 73, which may be integral with outer membrane 72 closes the chamber. Alternately, end cap 73 may be constructed to elute a predetermined medication, such as, for example, steroids. Steroids, such as dexamethasone sodium phosphate, beclamethasone, and the like, are used to control inflammatory processes.

In this arrangement, prior to inserting the catheter distal end into the patient's heart, the inner chamber 68 is charged with the genetic material which is to be dispensed into the myocardium. This may be done, for example, by simply inserting a micro needle through end cap 73, and inserting the desired bolus of genetic material into chamber 68. After the chamber 68 is filled and the catheter is implanted, body fluids will enter chamber 66 through membrane 72 to impart a pressure on the inner chamber 68 via the impermeable membrane 67. This results in a dispensing of the genetic material stored within chamber 68 through the lumen 74L of extension 74E and through the outlet 75 of the helical element 44. Although the preferred needle or element 44 is helical, additional configurations of needles or elements can also be used as known to those skilled in the art.

Still referring now to Fig. 4, there is illustrated another embodiment of a catheter tip useful for delivering a small bolus of the selected genetic material. In this embodiment, the bolus of material is stored within

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the hollow interior of distal needle 44, i.e., the interior is the reservoir. The interior reservoir is maintained sealed by use of a soluble material which is normally solid, but which dissolves when subjected to body fluids for a period of time. An example of such material is mannitol. Plugs or globules 81-85 of mannitol are illustrated (by dashed lines) in place to block the two ends of element 44, as well as the ports 76, 77, 78. This may be combined with an osmotic pump, as described in connection with Fig. 3, where the outer chamber is filled with a saline solution which forces the genetic material out of the ports of element 44. Another alternate embodiment, not shown, is to use a stylet which inserted through to the distal end of the catheter, to push a piston which aids in expressing the genetic material into the myocardial cells. Alternatively, the piston can be driven by a micro pump. In another embodiment, the genetic material contacts the myocardial cells by passive delivery.

Referring now to Fig. 5A, there is shown, by way of illustration, another embodiment of an implantable delivery system comprising a combined pacing lead and delivery catheter, hereinafter referred to simply as a catheter. In this embodiment, the catheter 90 is combined with a pacemaker or pulse generator (not shown) and a source of genetic material such as illustrated by pump 92 which is suitably implanted near the pacemaker. The proximal end 91 of the catheter is connected to the pacemaker in the standard fashion. The genetic material is delivered through connecting tube 93 to a proximal section 88 of the catheter, communicating with lengthwise catheter lumen illustrated at 89. Alternately, the pacemaker head may contain a reservoir and micropump, for providing delivery of the genetic material directly to the lumen 89. The main length of the catheter has an outside sheath of biocompatible insulating material 96, and at least one conductor coil 95 which communicates electrically from the pacemaker to electrode 97 at the distal tip of the catheter. The catheter further

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comprises an axially positioned polymeric cannula 94, having lumen 87, through at least a portion of the catheter length and positioned within coil 95, which provides an inner surface for the catheter lumen. The cannula terminates at the distal end of the catheter, just proximal to the tip portion of electrode 97, which is illustrated as having an outer porous surface. Electrode 97 has a central opening, shown covered with the porous electrode material, through which genetic material can pass when the catheter is positioned in the patient. As shown, conductor coil 95 is electrically connected to electrode 97, and connects pace pulses and sensed cardiac signals between the pacemaker and the electrode. Of course, for a bipolar embodiment, the lead/catheter 90 carries a second electrode (not shown), suitably a ring electrode just proximal to electrode 97. Also, as illustrated, a fixation mechanism such as tines 98 are employed for fixing or anchoring the distal tip to the heart wall of the patient.

In one embodiment, pump 92 is suitably an osmotic minipump, which pumps fluid contained within through tube 93, into catheter portion 88 and through the lumens 89, 87 to the tip electrode 97. As mentioned previously, the reservoir and pump may alternately be mounted in the pacemaker device itself. In either instance, the genetic material is delivered under very minimal pressure from the reservoir through the lumen of the catheter to the electrode, where it is passed through the electrode central channel to contact myocardial cells. In yet another embodiment, the lumen portion 87 provided by the cannula is utilized as the reservoir. In this embodiment, delivery may either be passive, or with the aid of a micropump (not shown). The genetic material can be preloaded into the cannula, or it can be inserted by a needle just before the catheter is introduced and positioned with the patient.

In another embodiment, as illustrated in Figure 5B, a chamber 99 is provided just proximal from eluting electrode 97, and serves as the reservoir of the genetic

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material. Insulating material 96 is formed from a self-sealing material such that it may be pierced with a needle, or the like, and reseal itself, thus allowing introduction of the genetic material into the chamber prior to  
5 implantation. Alternately, insulating material 96 can contain a port (not shown) through which the needle inserts the genetic material. In this embodiment, delivery of the material is without a pump, i.e., passive, the material draining slowly through the microporous portion of electrode  
10 97.

The above described delivery systems can be used, for example, in methods of pacing and enhancing the detectability of sensed cardiac signals. A supply of a genetic material of the class having the property of  
15 increasing the expression of ion channels in cardiac cells to which it is delivered is selected. A transvenous catheter, having proximal and distal ends and a pacing electrode at the distal end, is introduced into the patient. The distal end of the catheter is positioned against the  
20 patient's heart wall and the genetic material is delivered through the catheter and out of the distal end, to the cardiac cells adjacent the pacing electrode, thereby enhancing cardiac signals produced by the cells. Normal cardiac pacing is carried out with the pacemaker and  
25 connected catheter implanted in the patient.

Although a transvenous form of delivery system is preferred, it is to be understood that the invention can employ other methods and devices. For example, a small bolus of selected genetic material can be loaded into a  
30 micro-syringe, e.g., a 100  $\mu$ l Hamilton syringe, and applied directly from the outside of the heart.

As used herein, the phrase "cardiac signal" refers to any cardiac signal that is detectable and includes, but is not limited to, the P-wave.

35 As used herein, the phrase "signal to noise ratio" refers to the ratio of the amplitude of the cardiac signal, such as, for example, the P-wave, to the amplitude of the

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"noise." In addition, the signal to noise ratio can be measured for other cardiac signals as well. Sources of "noise" include, but are not limited to, the QRS complex and muscle noise. It is desirable to establish a high signal to noise ratio, i.e., a signal to noise ratio of greater than 1:1 for unipolar leads and greater than 3:1 for bipolar leads. It is even more preferred to establish a signal to noise ratio greater than 10:1.

As used herein, the phrase "ion channel protein genetic material" refers to recombinant nucleic acid molecules encoding an ion channel protein or, alternatively, an ion channel protein itself, which is used in the methods and delivery systems of the invention. For chronic treatment, or long term treatment, the ion channel protein genetic material will be in the form of recombinant nucleic acid molecules encoding the ion channel protein. In contrast, for acute treatment, or short term treatment, the ion channel protein genetic material will be in the form of the ion channel proteins themselves.

A "recombinant nucleic acid molecule", as used herein, is comprised of an isolated ion channel protein-encoding nucleotide sequence inserted into a delivery vehicle. Regulatory elements, such as the promoter and polyadenylation signal, are operably linked to the nucleotide sequence encoding the ion channel protein, whereby the protein is capable of being produced when the recombinant nucleic acid molecule is introduced into a cell.

The nucleic acid molecules encoding the ion channel proteins are prepared synthetically or, preferably, from isolated nucleic acid molecules, as described below. A nucleic acid is "isolated" when purified away from other cellular constituents, such as, for example, other cellular nucleic acids or proteins, by standard techniques known to those of ordinary skill in the art. The coding region of the nucleic acid molecule encoding the ion channel protein can encode a full length gene product or a subfragment thereof, or a novel mutated or fusion sequence. The protein

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coding sequence can be a sequence endogenous to the target cell, or exogenous to the target cell. The promoter, with which the coding sequence is operably associated, may or may not be one that normally is associated with the coding  
5 sequence.

The nucleic acid molecule encoding the ion channel protein is inserted into an appropriate delivery vehicle, such as, for example, an expression plasmid, cosmid, YAC vector, and the like. Almost any delivery vehicle can be  
10 used for introducing nucleic acids into the cardiovascular system, including, for example, recombinant vectors, such as one based on adenovirus serotype 5, Ad5, as set forth in French, et al., *Circulation*, 1994, 90, 2414-2424, which is incorporated herein by reference. An additional protocol  
15 for adenovirus-mediated gene transfer to cardiac cells is set forth in WO 94/11506, Johns, J. *Clin. Invest.*, 1995, 96, 1152-1158, and in Barr, et al., *Gene Ther.*, 1994, 1, 51-58, both of which are incorporated herein by reference. Other recombinant vectors include, for example, plasmid DNA  
20 vectors, such as one derived from pGEM3 or pBR322, as set forth in Acsadi, et al., *The New Biol.*, 1991, 3, 71-81, and Gal, et al., *Lab. Invest.*, 1993, 68, 18-25, both of which are incorporated herein by reference, cDNA-containing liposomes, artificial viruses, nanoparticles, and the like.  
25 It is also contemplated that ion channel proteins be injected directly into the myocardium.

The regulatory elements of the recombinant nucleic acid molecules of the invention are capable of directing expression in mammalian cells, specifically human cells.  
30 The regulatory elements include a promoter and a polyadenylation signal. In addition, other elements, such as a Kozak region, may also be included in the recombinant nucleic acid molecule. Examples of polyadenylation signals useful to practice the present invention include, but are  
35 not limited to, SV40 polyadenylation signals and LTR polyadenylation signals. In particular, the SV40 polyadenylation signal which is in pCEP4 plasmid

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(Invitrogen, San Diego, CA), referred to as the SV40 polyadenylation signal, can be used.

The promoters useful in constructing the recombinant nucleic acid molecules of the invention may be constitutive or inducible. A constitutive promoter is expressed under all conditions of cell growth. Exemplary constitutive promoters include the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase,  $\beta$ -actin, human myosin, human hemoglobin, human muscle creatine, and others. In addition, many viral promoters function constitutively in eukaryotic cells, and include, but are not limited to, the early and late promoters of SV40, the Mouse Mammary Tumor Virus (MMTV) promoter, the long terminal repeats (LTRs) of Maloney leukemia virus, Human Immunodeficiency Virus (HIV), Cytomegalovirus (CMV) immediate early promoter, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV), and other retroviruses, and the thymidine kinase promoter of herpes simplex virus. Other promoters are known to those of ordinary skill in the art.

Inducible promoters are expressed in the presence of an inducing agent. For example, the metallothionein promoter is induced to promote (increase) transcription in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

Promoters and polyadenylation signals used must be functional within the cells of the mammal. In order to maximize protein production, regulatory sequences may be selected which are well suited for gene expression in the cardiac cells into which the recombinant nucleic acid molecule is administered. For example, the promoter is preferably a cardiac tissue-specific promoter-enhancer, such as, for example, cardiac isoform troponin C (cTNC) promoter. Parmacek, et al., *J. Biol. Chem.*, 1990, 265, 15970-15976, and Parmacek, et al., *Mol. Cell Biol.*, 1992, 12, 1967-1976. In addition, codons may be selected which are most efficiently transcribed in the cell. One having ordinary

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skill in the art can produce recombinant nucleic acid molecules which are functional in the cardiac cells.

Genetic material can be introduced into a cell or "contacted" by a cell by, for example, transfection or  
5 transduction procedures. Transfection refers to the acquisition by a cell of new genetic material by incorporation of added nucleic acid molecules. Transfection can occur by physical or chemical methods. Many transfection techniques are known to those of ordinary skill  
10 in the art including: calcium phosphate DNA co-precipitation; DEAE-dextran DNA transfection; electroporation; naked plasmid adsorption, and cationic liposome-mediated transfection. Transduction refers to the process of transferring nucleic acid into a cell using a DNA  
15 or RNA virus. Suitable viral vectors for use as transducing agents include, but are not limited to, retroviral vectors, adeno associated viral vectors, vaccinia viruses, and Semliki Forest virus vectors.

Treatment of cells, or contacting cells, with  
20 recombinant nucleic acid molecules can take place *in vivo* or *ex vivo*. For *ex vivo* treatment, cells are isolated from an animal (preferably a human), transformed (*i.e.*, transduced or transfected *in vitro*) with a delivery vehicle containing a nucleic acid molecule encoding an ion channel protein, and  
25 then administered to a recipient. Procedures for removing cells from mammals are well known to those of ordinary skill in the art. In addition to cells, tissue or the whole or parts of organs may be removed, treated *ex vivo* and then returned to the patient. Thus, cells, tissue or organs may  
30 be cultured, bathed, perfused and the like under conditions for introducing the recombinant nucleic acid molecules of the invention into the desired cells.

For *in vivo* treatment, cells of an animal, preferably a mammal and most preferably a human, are  
35 transformed *in vivo* with a recombinant nucleic acid molecule of the invention. The *in vivo* treatment may involve systemic intravenous treatment with a recombinant nucleic



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acid molecule, local internal treatment with a recombinant nucleic acid molecule, such as by localized perfusion or topical treatment, and the like. When performing *in vivo* administration of the recombinant nucleic acid molecule, the preferred delivery vehicles are based on noncytopathic eukaryotic viruses in which nonessential or complementable genes have been replaced with the nucleic acid sequence of interest. Such noncytopathic viruses include retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have recently been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (*i.e.*, capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for high-efficiency transduction of genes *in vivo*. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell line with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, M. "Gene Transfer and Expression, a Laboratory Manual", W.H. Freeman Co., New York (1990) and Murry, E.J. e.d. "Methods in Molecular Biology", Vol. 7, Humana Press, Inc., Clifton, New Jersey (1991).

A preferred virus for contacting cells in certain applications, such as in *in vivo* applications, is the adeno-associated virus, a double-stranded DNA virus. The adeno-associated virus can be engineered to be replication deficient and is capable of infecting a wide range of cell types and species. It further has advantages such as heat and lipid solvent stability, high transduction frequencies in cells of diverse lineages, including hemopoietic cells, and lack of superinfection inhibition thus allowing multiple

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series of transductions. Recent reports indicate that the adeno-associated virus can also function in an extrachromosomal fashion.

In preferred embodiments of the present invention, the recombinant nucleic acid molecules comprising nucleic acid molecules encoding the ion channel proteins, or, in the alternative, the ion channel proteins, are delivered to cardiac cells adjacent the atrial or ventricular electrode, or both, using the delivery systems set forth above.

10 Alternatively, the ion channel protein genetic material is delivered to the cardiac cells by direct injection.

In preferred embodiments of the present invention, the nucleic acid molecules encoding the ion channel proteins comprise the full length coding sequence cDNA of an ion channel protein. Preferably, the ion channel proteins are sodium channel proteins; more preferably, the ion channel protein is the voltage-regulated sodium channel hH1. Such a nucleic acid molecule is described in the Gellens, et al., *Proc. Natl. Acad. Sci. USA*, 1992, 89, 554-558, and White, et al., *Mol. Pharmacol.*, 1991, 39, 604-608 references, both of which are incorporated herein by reference, which contain the full length amino acid sequence and cDNA sequence, respectively.

Introduction of the ion channel-encoding nucleic acid molecules or the ion channel proteins to cardiac cells adjacent the atrial or ventricular electrode will result in increased expression of sodium channels, producing a larger cardiac signal, such as, for example, P-wave, and thus, an improved or corrected signal to noise ratio.

30 Nucleic acid molecules comprising nucleotide sequences encoding hH1 sodium channel are isolated and purified according to the methods set forth in Gellens, et al., *Proc. Natl. Acad. Sci. USA*, 1992, 89, 554-558, and White, et al., *Mol. Pharmacol.*, 1991, 39, 604-608. The nucleic acid and protein sequences of hH1 sodium channel are set forth in SEQ ID NO:1 and SEQ ID NO:2, respectively. It is contemplated that nucleic acid molecules comprising

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nucleotide sequences that are preferably at least 70% homologous, more preferably at least 80% homologous, and most preferably at least 90% homologous to the ion channel nucleotide sequences described in SEQ ID NO:1 can also be  
5 used.

It is understood that minor modifications of nucleotide sequence or the primary amino acid sequence may result in proteins which have substantially equivalent or enhanced activity as compared to the ion channel proteins  
10 exemplified herein. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental such as through mutations in hosts which produce the ion channel proteins. A "mutation" in a protein alters its primary structure (relative to the commonly occurring or  
15 specifically described protein) due to changes in the nucleotide sequence of the DNA which encodes it. These mutations specifically include allelic variants. Mutational changes in the primary structure of a protein can result from deletions, additions, or substitutions. A "deletion"  
20 is defined as a polypeptide in which one or more internal amino acid residues are absent as compared to the native sequence. An "addition" is defined as a polypeptide which has one or more additional internal amino acid residues as compared to the wild type protein. A "substitution" results  
25 from the replacement of one or more amino acid residues by other residues. A protein "fragment" is a polypeptide consisting of a primary amino acid sequence which is identical to a portion of the primary sequence of the protein to which the polypeptide is related.

30 Preferred "substitutions" are those which are conservative, i.e., wherein a residue is replaced by another of the same general type. As is well understood, naturally-occurring amino acids can be subclassified as acidic, basic, neutral and polar, or neutral and nonpolar and/or aromatic.  
35 It is generally preferred that encoded peptides differing from the native form contain substituted codons for amino acids which are from the same group as that of the amino

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acid replaced. Thus, in general, the basic amino acids Lys, Arg, and Histidine are interchangeable; the acidic amino acids Asp and Glu are interchangeable; the neutral polar amino acids Ser, Thr, Cys, Gln, and Asn are interchangeable; 5 the nonpolar aliphatic acids Gly, Ala, Val, Ile, and Leu are conservative with respect to each other (but because of size, Gly and Ala are more closely related and Val, Ile and Leu are more closely related), and the aromatic amino acids Phe, Trp, and Tyr are interchangeable.

10 While Pro is a nonpolar neutral amino acid, it represents difficulties because of its effects on conformation, and substitutions by or for Pro are not preferred, except when the same or similar conformational results can be obtained. Polar amino acids which represent 15 conservative changes include Ser, Thr, Gln, Asn; and to a lesser extent, Met. In addition, although classified in different categories, Ala, Gly, and Ser seem to be interchangeable, and Cys additionally fits into this group, or may be classified with the polar neutral amino acids. 20 Some substitutions by codons for amino acids from different classes may also be useful.

Once the nucleic acid molecules encoding the ion channel proteins are isolated and purified according to the methods described above, recombinant nucleic acid molecules 25 are prepared in which the desired ion channel nucleic acid molecule is incorporated into a delivery vehicle by methods known to those skilled in the art, as taught in, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989). 30 Preferred delivery vehicles include, for example, plasmids (Acsadi, et al., *The New Biol.*, 1991, 3, 71-81, and Gal, et al., *Lab. Invest.*, 1993, 68, 18-25, both of which are incorporated herein by reference) and adenovirus (WO 94/11506, Johns, *J. Clin. Invest.*, 1995, 96, 1152-1158, and 35 in Barr, et al., *Gene Ther.*, 1994, 1, 51-58, each of which are incorporated herein by reference). The nucleic acid molecules encoding ion channel proteins, or ion channel

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proteins produced therefrom, are delivered to the cardiac cells adjacent to the atrial electrode by the delivery systems of the present invention. Thus, such delivery systems of the present invention are used to contact the  
5 cardiac cells adjacent the atrial electrode with recombinant nucleic acid molecules encoding an ion channel protein, or ion channel proteins.

Where the ion channel protein genetic material is in the form of ion channel proteins, such proteins can be  
10 prepared in large quantities by using various standard expression systems known to those skilled in the art. Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989), pp. 16.1-16.55, incorporated herein by reference.

15 The recombinant nucleic acid molecules or ion channel proteins are preferably delivered in a pharmaceutical composition. Such pharmaceutical compositions can include, for example, the recombinant nucleic acid molecule or protein in a volume of phosphate-  
20 buffered saline with 5% sucrose. In other embodiments of the invention, the recombinant nucleic acid molecule or protein is delivered with suitable pharmaceutical carriers, such as those described in the most recent edition of *Remington's Pharmaceutical Sciences*, A. Osol, a standard  
25 reference text in this field. The recombinant nucleic acid molecule or protein is delivered in a therapeutically effective amount. Such amount is determined experimentally and is that amount which either improves or corrects the P-wave signal to noise ratio by enhancing the P-wave amplitude  
30 as a result of the increased expression of sodium channels in the cardiac cells adjacent the atrial or ventricular electrode. The amount of recombinant nucleic acid molecule or protein is preferably between 0.01  $\mu$ g and 100 mg, more preferably between 0.1  $\mu$ g and 10 mg, more preferably between  
35 1  $\mu$ g and 1 mg, and most preferably between 10  $\mu$ g and 100  $\mu$ g. A single therapeutically effective amount is referred to as a bolus. Where adenovirus vectors are used, the amount of

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recombinant nucleic acid molecule is preferably between  $10^7$  plaque forming units (pfu) and  $10^{15}$  pfu, more preferably between  $10^8$  pfu and  $10^{14}$  pfu, and most preferably between  $10^9$  pfu and  $10^{12}$  pfu. A single therapeutically effective amount  
5 of ion channel protein genetic material is referred to as a bolus. In some embodiments of the present invention, the delivery of the recombinant nucleic acid molecules or proteins is combined with steroid elution, such as with dexamethasone sodium phosphate, beclamethasone, and the  
10 like, to control inflammatory processes.

In some embodiments of the invention, it may be preferred to administer, in addition to ion channel protein genetic material, delivery vehicle encoding the Na<sup>+</sup>/K<sup>+</sup> pump. The Na<sup>+</sup>/K<sup>+</sup> pump acts to discharge Na<sup>+</sup> ions from the myocardial  
15 cells that have accumulated as a result of the introduction of the ion channel protein genetic material. This treatment can be optional, as determined by the skilled practitioner. cDNA encoding the alpha and beta subunits of the human Na<sup>+</sup>/K<sup>+</sup> pump are set forth in Kawakami, et al., *J. Biochem.*, 1986,  
20 100, 389-397, and Kawakami, et al., *Nuc. Acids Res.*, 1986, 14, 2833-2844, both of which are incorporated herein by reference. The nucleic acid and amino acid sequences for the alpha subunit are set forth in SEQ ID NO:5 and SEQ ID NO:6, respectively. The nucleic acid and amino acid  
25 sequences for the beta subunit are set forth in SEQ ID NO:7 and SEQ ID NO:8, respectively. The delivery vehicles for the pump subunits can be constructed from cDNA libraries in the same manner as set forth for hH1, except that the forward primer 5'-ATGGGGAAGGGGGTTGGACGTGAT-3' (SEQ ID NO:9)  
30 and reverse primer 5'-ATAGTAGGTTTCCTTCTCCACCCA-3' (SEQ ID NO:10) for the alpha subunit, and the forward primer 5'-ATGGCCCGCGGGAAGCCAAGGAG-3' (SEQ ID NO:11) and reverse primer 5'-GCTCTTAACTTCAATTTTACATC-3' (SEQ ID NO:12) for the beta subunit are used. It is understood that other primers can  
35 be used in addition to those set forth herein, as is well known to the skilled artisan. A therapeutically effective amount of the genetic material encoding the Na<sup>+</sup>/K<sup>+</sup> pump is

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delivered to the myocardial cells using the delivery systems described herein. The therapeutically effective amount is determined by the practitioner, and depends upon the results achieved with the ion channel protein genetic material.

5 In preferred embodiments of the invention, the recombinant nucleic acid molecules encoding the ion channel proteins is delivered with class I and/or class IV antiarrhythmic drugs, such as, for example, verapamil, mexiletine, and the like, or combinations thereof. These  
10 drugs may be delivered subcutaneously, intravenously, injected in the immediate vicinity of the atrial electrode, or as determined by the skilled artisan. These drugs may be delivered by one injection, or in multiple injections. The amount of antiarrhythmic drugs depends upon the age, weight,  
15 sex, and other characteristics of the patient, and is determined empirically by the skilled artisan. Class I and/or class IV antiarrhythmic drugs have been shown to enhance sodium ion channel expression in mammals. Duff, et al., *Mol. Pharmacol.*, 1992, 42, 570-574, and Taouis, et al.,  
20 *J. Clin. Invest.*, 1991, 88, 375-378, both of which are incorporated herein by reference.

The following examples are meant to be exemplary of the preferred embodiments of the invention and are not meant to be limiting.

## 25 EXAMPLES

### Example 1: Isolation and Purification of Nucleic Acid Molecule Encoding hH1

Nucleic acid molecules encoding hH1 are isolated and purified according to general methods well known to  
30 those skilled in the art, and in particular, by the method set forth in Gellens, et al., *Proc. Natl. Acad. Sci. USA*, 1992, 89, 554-558, incorporated herein by reference. Briefly, a size selected and random-primed adult human cardiac cDNA library constructed in  $\lambda$ ZAPII (Stratagene) is  
35 screened with cDNA probes corresponding to nucleotides 1-4385 and 5424-7076 derived from the rat muscle TTX-I isoform (rSkM2), as set forth in Kallen, et al., *Neuron*, 1990, 4,

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233-242, incorporated herein by reference. Hybridizations are performed at 42°C for 18 hours in 50% formamide, 5X SSPE, 5X Denhardt's solution, 0.1% SDS/salmon sperm DNA, random primed <sup>32</sup>P-labeled probe. Filters are washed with 6X standard saline citrate, 0.1% SDS at 65°C. Plaque purified clones are rescued as pBluescript phagemids and sequenced as described in Kallen, et al., *Neuron*, 1990, 4, 233-242. A full-length hH1 construct is made in pBluescript by sequential ligation of S14 *EcoRI*-*Sac II* (nt +1 to +252), C75 *Sac II*-*KpnI* (nt +253 to +4377), and C92 *KpnI*-*EcoRI* (nt +4378 to +8491) fragments and the full length insert is moved into a modified pSP64T vector, as set forth in White, et al., *Mol. Pharmacol.*, 1991, 39, 604-608, incorporated herein by reference. Nucleotides -151 to -8 of the 5' untranslated region are deleted from the construct using exonuclease III and mung bean nuclease, as set forth in White, et al., *Mol. Pharmacol.*, 1991, 39, 604-608.

Alternatively, cDNA for hH1 may be prepared from fresh cardiac tissue. Briefly, total cellular RNA is isolated and purified (Chomczynsky, et al., *Anal. Biochem.*, 1987, 162, 156-159) from heart tissue, obtained from cardiac transplantation donors, or from salvaged tissue, and selected for poly(A) RNA (Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989), pp. 7.26-7.29). cDNA corresponding to the hH1 sodium channel protein is prepared from the poly(A) cardiac RNA by reverse transcription using a GENEAMP™ PCR kit (Perkin Elmer Cetus, Norwalk, CT), or the like, using random hexamers according to the manufacturer's instructions. The specific hH1 nucleic acid molecules are amplified by the polymerase chain reaction (PCR), also using the GENEAMP™ PCR kit, or the like, using forward and reverse primers specific for hH1 according to the manufacturer's instructions. For example, the forward primer for cloning hH1 is preferably 5'-ATGGCAAACCTTCCTATTACCTCGG-3' (SEQ ID NO:3), and the reverse primer is 5'-CACGATGGACTCACGGTCCCTGTC-3' (SEQ ID NO:4). It is understood that additional primers can be used



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for amplification as determined by those skilled in the art. These primers may be preceded at the 5' terminus by nucleotide sequences containing endonuclease restriction sites for easy incorporation into vectors. The specific ion channel nucleic acid molecules can also be amplified by PCR from human genomic DNA (Stratagene, San Diego, CA). After cutting the PCR products with the appropriate restriction endonuclease(s), the PCR products are purified by phenol:chloroform extractions, or using commercial purification kits, such as, for example, MAGIC™ Minipreps DNA Purification System (Promega, Madison, WI). The specific nucleotide sequence of the PCR products is determined by conventional DNA sequencing procedures, and the identity of the PCR products confirmed by comparison to the published sequences for the ion channel proteins.

#### Example 2: Insertion of Ion Channel cDNA into Delivery Vehicles

Preferably, ion channel cDNA is inserted into either plasmid or adenoviral vectors. Plasmid vectors include for example, pGEM3 or pBR322, as set forth in Acsadi, et al., *The New Biol.*, 1991, 3, 71-81, and Gal, et al., *Lab. Invest.*, 1993, 68, 18-25. Adenoviral vectors include for example, adenovirus serotype 5, Ad5, as set forth in French, et al., *Circulation*, 1994, 90, 2414-2424, and Johns, *J. Clin. Invest.*, 1995, 96, 1152-1158.

Preferably, the primers used to amplify the ion channel nucleic acid molecules are designed with unique endonuclease restriction sites located at the 5' terminus. In the absence of such design, polylinker arms, containing unique restriction sites, can be ligated thereto. After cutting the purified PCR products with the appropriate restriction endonuclease(s), the plasmid vector, comprising a polylinker, is also cut with the same restriction endonuclease(s), affording the ion channel nucleic acid molecule a site at which to ligate. In a similar manner, recombinant adenovirus (Gluzman, et al., in *Eukaryotic Viral Vectors*, Gluzman, ed., Cold Spring Harbor Press, 1982,

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pp.187-192, French, et al., *Circulation*, 1994, 90, 2414-2424, and Johns, J. *Clin. Invest.*, 1995, 96, 1152-1158) containing ion channel cDNA molecules are prepared in accordance with standard techniques well known to those  
5 skilled in the art.

It is contemplated that variations of the above-described invention may be constructed that are consistent with the spirit of the invention.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANTS: Ken Stokes  
Josée Morissette
- (ii) TITLE OF INVENTION: SYSTEMS FOR ENHANCING CARDIAC SIGNAL  
SENSING BY CARDIAC PACEMAKERS THROUGH  
GENETIC TREATMENT
- (iii) NUMBER OF SEQUENCES: 12
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Woodcock Washburn Kurtz Mackiewicz & Norris LLP
  - (B) STREET: One Liberty Place - 46th Floor
  - (C) CITY: Philadelphia
  - (D) STATE: PA
  - (E) COUNTRY: U.S.A.
  - (F) ZIP: 19103
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: WordPerfect 6.1
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: N/A
  - (B) FILING DATE: Herewith
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Paul K. Legaard
  - (B) REGISTRATION NUMBER: 38,534
  - (C) REFERENCE/DOCKET NUMBER: MEDT-0082
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (215) 568-3100
  - (B) TELEFAX: (215) 568-3439

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 6048 bases
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
 

ATG GCA AAC TTC CTA TTA CCT CGG GGC ACC AGC AGC TTC CGC AGG	45
Met Ala Asn Phe Leu Leu Pro Arg Gly Thr Ser Ser Phe Arg Arg	15
1 5 10	
TTC ACA CGG GAG TCC CTG GCA GCC ATC GAG AAG CGC ATG GCG GAG	90
Phe Thr Arg Glu Ser Leu Ala Ala Ile Glu Lys Arg Met Ala Glu	30
20 25 30	
AAG CAA GCC CGC GGC TCA ACC ACC TTG CAG GAG AGC CGA GAG GGG	135
Lys Gln Ala Arg Gly Ser Thr Thr Leu Gln Glu Ser Arg Glu Gly	45
35 40 45	
CTG CCC GAG GAG GAG GCT CCC CGG CCC CAG CTG GAC CTG CAG GCC	180
Leu Pro Glu Glu Glu Ala Pro Arg Pro Gln Leu Asp Leu Gln Ala	60
50 55 60	

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TCC AAA AAG CTG CCA GAT CTC TAT GGC AAT CCA CCC CAA GAG CTC	225
Ser Lys Lys Leu Pro Asp Leu Tyr Gly Asn Pro Pro Gln Glu Leu	
65 70 75	
ATC GGA GAG CCC CTG GAG GAC CTG GAC CCC TTC TAT AGC ACC CAA	270
Ile Gly Glu Pro Leu Glu Asp Leu Asp Pro Phe Tyr Ser Thr Gln	
80 85 90	
AAG ACT TTC ATC GTA CTG AAT AAA GGC AAG ACC ATC TTC CGG TTC	315
Lys Thr Phe Ile Val Leu Asn Lys Gly Lys Thr Ile Phe Arg Phe	
95 100 105	
AGT GCC ACC AAC GCC TTG TAT GTC CTC AGT CCC TTC CAC CCA GTT	360
Ser Ala Thr Asn Ala Leu Tyr Val Leu Ser Pro Phe His Pro Val	
110 115 120	
CGG AGA GCG GCT GTG AAG ATT CTG GTT CAC TCG CTC TTC AAC ATG	405
Arg Arg Ala Ala Val Lys Ile Leu Val His Ser Leu Phe Asn Met	
125 130 135	
CTC ATC ATG TGC ACC ATC CTC ACC AAC TGC GTG TTC ATG GCC CAG	450
Leu Ile Met Cys Thr Ile Leu Thr Asn Cys Val Phe Met Ala Gln	
140 145 150	
CAC GAC CCT CCA CCC TGG ACC AAG TAT GTC GAG TAC ACC TTC ACC	495
His Asp Pro Pro Pro Trp Thr Lys Tyr Val Glu Tyr Thr Phe Thr	
155 160 165	
GCC ATT TAC ACC TTT GAG TCT CTG GTC AAG ATT CTG GCT CGA GCT	540
Ala Ile Tyr Thr Phe Glu Ser Leu Val Lys Ile Leu Ala Arg Ala	
170 175 180	
TTC TGC CTG CAC GCG TTC ACT TTC CTT CGG GAC CCA TGG AAC TGG	585
Phe Cys Leu His Ala Phe Thr Phe Leu Arg Asp Pro Trp Asn Trp	
185 190 195	
CTG GAC TTT AGT GTG ATT ATC ATG GCA TAC ACA ACT GAA TTT GTG	630
Leu Asp Phe Ser Val Ile Ile Met Ala Tyr Thr Thr Glu Phe Val	
200 205 210	
GAC CTG GGC AAT GTC TCA GCC TTA CGC ACC TTC CGA GTC CTC CGG	675
Asp Leu Gly Asn Val Ser Ala Leu Arg Thr Phe Arg Val Leu Arg	
215 220 225	
GCC CTG AAA ACT ATA TCA GTC ATT TCA GGG CTG AAG ACC ATC GTG	720
Ala Leu Lys Thr Ile Ser Val Ile Ser Gly Leu Lys Thr Ile Val	
230 235 240	
GGG GCC CTG ATC CAG TCT GTG AAG AAG CTG GCT GAT GTG ATG GTC	765
Gly Ala Leu Ile Gln Ser Val Lys Lys Leu Ala Asp Val Met Val	
245 250 255	
CTC ACA GTC TTC TGC CTC AGC GTC TTT GCC CTC ATC GGC CTG CAG	810
Leu Thr Val Phe Cys Leu Ser Val Phe Ala Leu Ile Gly Leu Gln	
260 265 270	
CTC TTC ATG GGC AAC CTA AGG CAC AAG TGT GTG CGC AAC TTC ACA	855
Leu Phe Met Gly Asn Leu Arg His Lys Cys Val Arg Asn Phe Thr	
275 280 285	
GCG CTC AAC GGC ACC AAC GGC TCC GTG GAG GCC GAC GGC TTG GTC	900
Ala Leu Asn Gly Thr Asn Gly Ser Val Glu Ala Asp Gly Leu Val	
290 295 300	
TGG GAA TCC CTG GAC CTT TAC CTC AGT GAT CCA GAA AAT TAC CTG	945
Trp Glu Ser Leu Asp Leu Tyr Leu Ser Asp Pro Glu Asn Tyr Leu	
305 310 315	

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CTC	AAG	AAC	GGC	ACC	TCT	GAT	GTG	TTA	CTG	TGT	GGG	AAC	AGC	TCT	990
Leu	Lys	Asn	Gly	Thr	Ser	Asp	Val	Leu	Leu	Cys	Gly	Asn	Ser	Ser	
				320					325					330	
GAC	GCT	GGG	ACA	TGT	CCG	GAG	GGC	TAC	CGG	TGC	CTA	AAG	GCA	GGC	1035
Asp	Ala	Gly	Thr	Cys	Pro	Glu	Gly	Tyr	Arg	Cys	Leu	Lys	Ala	Gly	
				335					340					345	
GAG	AAC	CCC	GAC	CAC	GGC	TAC	ACC	AGC	TTC	GAT	TCC	TTT	GCC	TGG	1080
Glu	Asn	Pro	Asp	His	Gly	Tyr	Thr	Ser	Phe	Asp	Ser	Phe	Ala	Trp	
				350					355					360	
GCC	TTT	CTT	GCA	CTC	TTC	CGC	CTG	ATG	ACG	CAG	GAC	TGC	TGG	GAG	1125
Ala	Phe	Leu	Ala	Leu	Phe	Arg	Leu	Met	Thr	Gln	Asp	Cys	Trp	Glu	
				365					370					375	
CGC	CTC	TAT	CAG	CAG	ACC	CTC	AGG	TCC	GCA	GGG	AAG	ATC	TAC	ATG	1170
Arg	Leu	Tyr	Gln	Gln	Thr	Leu	Arg	Ser	Ala	Gly	Lys	Ile	Tyr	Met	
				380					385					390	
ATC	TTC	TTC	ATG	CTT	GTC	ATC	TTC	CTG	GGG	TCC	TTC	TAC	CTG	GTG	1215
Ile	Phe	Phe	Met	Leu	Val	Ile	Phe	Leu	Gly	Ser	Phe	Tyr	Leu	Val	
				395					400					405	
AAC	CTG	ATC	CTG	GCC	GTG	GTC	GCA	ATG	GCC	TAT	GAG	GAG	CAA	AAC	1260
Asn	Leu	Ile	Leu	Ala	Val	Val	Ala	Met	Ala	Tyr	Glu	Glu	Gln	Asn	
				410					415					420	
CAA	GCC	ACC	ATC	GCT	GAG	ACC	GAG	GAG	AAG	GAA	AAG	CGC	TTC	CAG	1305
Gln	Ala	Thr	Ile	Ala	Glu	Thr	Glu	Glu	Lys	Glu	Lys	Arg	Phe	Gln	
				425					430					435	
GAG	GCC	ATG	GAA	ATG	CTC	AAG	AAA	GAA	CAC	GAG	GCC	CTC	ACC	ATC	1350
Glu	Ala	Met	Glu	Met	Leu	Lys	Lys	Glu	His	Glu	Ala	Leu	Thr	Ile	
				440					445					450	
AGG	GGT	GTG	GAT	ACC	GTG	TCC	CGT	AGC	TCC	TTG	GAG	ATG	TCC	CCT	1395
Arg	Gly	Val	Asp	Thr	Val	Ser	Arg	Ser	Ser	Leu	Glu	Met	Ser	Pro	
				455					460					465	
TTG	GCC	CCA	GTA	AAC	AGC	CAT	GAG	AGA	AGA	AGC	AAG	AGG	AGA	AAA	1440
Leu	Ala	Pro	Val	Asn	Ser	His	Glu	Arg	Arg	Ser	Lys	Arg	Arg	Lys	
				470					475					480	
CGG	ATG	TCT	TCA	GGA	ACT	GAG	GAG	TGT	GGG	GAG	GAC	AGG	CTC	CCC	1485
Arg	Met	Ser	Ser	Gly	Thr	Glu	Glu	Cys	Gly	Glu	Asp	Arg	Leu	Pro	
				485					490					495	
AAG	TCT	GAC	TCA	GAA	GAT	GGT	CCC	AGA	GCA	ATG	AAT	CAT	CTC	AGC	1520
Lys	Ser	Asp	Ser	Glu	Asp	Gly	Pro	Arg	Ala	Met	Asn	His	Leu	Ser	
				500					505					510	
CTC	ACC	CGT	GGC	CTC	AGC	AGG	ACT	TCT	ATG	AAG	CCA	CGT	TCC	AGC	1565
Leu	Thr	Arg	Gly	Leu	Ser	Arg	Thr	Ser	Met	Lys	Pro	Arg	Ser	Ser	
				515					520					525	
CGC	GGG	AGC	ATT	TTC	ACC	TTT	CGC	AGG	CGA	GAC	CTG	GGT	TCT	GAA	1620
Arg	Gly	Ser	Ile	Phe	Thr	Phe	Arg	Arg	Arg	Asp	Leu	Gly	Ser	Glu	
				530					535					540	
GCA	GAT	TTT	GCA	GAT	GAT	GAA	AAC	AGC	ACA	GCG	CGG	GAG	AGC	GAG	1665
Ala	Asp	Phe	Ala	Asp	Asp	Glu	Asn	Ser	Thr	Ala	Arg	Glu	Ser	Glu	
				545					550					555	
AGC	CAC	CAC	ACA	TCA	CTG	CTG	GTG	CCC	TGG	CCC	CTG	CGC	CGG	ACC	1710
Ser	His	His	Thr	Ser	Leu	Leu	Val	Pro	Trp	Pro	Leu	Arg	Arg	Thr	
				560					565					570	

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AGT	GCC	CAG	GGA	CAG	CCC	AGT	CCC	GGA	ACC	TCG	GCT	CCT	GGC	CAC	1755
Ser	Ala	Gln	Gly	Gln	Pro	Ser	Pro	Gly	Thr	Ser	Ala	Pro	Gly	His	
				575					580					585	
GCC	CTC	CAT	GGC	AAA	AAG	AAC	AGC	ACT	GTG	GAC	TGC	AAT	GGG	GTG	1800
Ala	Leu	His	Gly	Lys	Lys	Asn	Ser	Thr	Val	Asp	Cys	Asn	Gly	Val	
				590					595					600	
GTC	TCA	TTA	CTG	GGG	GCA	GGC	GAC	CCA	GAG	GCC	ACA	TCC	CCA	GGA	1845
Val	Ser	Leu	Leu	Gly	Ala	Gly	Asp	Pro	Glu	Ala	Thr	Ser	Pro	Gly	
				605					610					615	
AGC	CAC	CTC	CTC	CGC	CCT	GTG	ATG	CTA	GAG	CAC	CCG	CCA	GAC	ACG	1890
Ser	His	Leu	Leu	Arg	Pro	Val	Met	Leu	Glu	His	Pro	Pro	Asp	Thr	
				620					625					630	
ACC	ACG	CCA	TCG	GAG	GAG	CCA	GGC	GGC	CCC	CAG	ATG	CTG	ACC	TCC	1935
Thr	Thr	Pro	Ser	Glu	Glu	Pro	Gly	Gly	Pro	Gln	Met	Leu	Thr	Ser	
				635					640					645	
CAG	GCT	CCG	TGT	GTA	GAT	GGC	TTC	GAG	GAG	CCA	GGA	GCA	CGG	CAG	1980
Gln	Ala	Pro	Cys	Val	Asp	Gly	Phe	Glu	Glu	Pro	Gly	Ala	Arg	Gln	
				650					655					660	
CGG	GCC	CTC	AGC	GCA	GTC	AGC	GTC	CTC	ACA	AGC	GCA	CTG	GAA	GAG	2025
Arg	Ala	Leu	Ser	Ala	Val	Ser	Val	Leu	Thr	Ser	Ala	Leu	Glu	Glu	
				665					670					675	
TTA	GAG	GAG	TCT	CGC	CAC	AAG	TGT	CCA	CCA	TGC	TGG	AAC	CGT	CTC	2070
Leu	Glu	Glu	Ser	Arg	His	Lys	Cys	Pro	Pro	Cys	Trp	Asn	Arg	Leu	
				680					685					690	
GCC	CAG	CGC	TAC	CTG	ATC	TGG	GAG	TGC	TGC	CCG	CTG	TGG	ATG	TCC	2115
Ala	Gln	Arg	Tyr	Leu	Ile	Trp	Glu	Cys	Cys	Pro	Leu	Trp	Met	Ser	
				695					700					705	
ATC	AAG	CAG	GGA	GTG	AAG	TTG	GTG	GTC	ATG	GAC	CCG	TTT	ACT	GAC	2160
Ile	Lys	Gln	Gly	Val	Lys	Leu	Val	Val	Met	Asp	Pro	Phe	Thr	Asp	
				710					715					720	
CTC	ACC	ATC	ACT	ATG	TGC	ATC	GTA	CTC	AAC	ACA	CTC	TTC	ATG	GCG	2205
Leu	Thr	Ile	Thr	Met	Cys	Ile	Val	Leu	Asn	Thr	Leu	Phe	Met	Ala	
				725					730					735	
CTG	GAG	CAC	TAC	AAC	ATG	ACA	AGT	GAA	TTC	GAG	GAG	ATG	CTG	CAG	2250
Leu	Glu	His	Tyr	Asn	Met	Thr	Ser	Glu	Phe	Glu	Glu	Met	Leu	Gln	
				740					745					750	
GTC	GGA	AAC	CTG	GTC	TTC	ACA	GGG	ATT	TTC	ACA	GCA	GAG	ATG	ACC	2295
Val	Gly	Asn	Leu	Val	Phe	Thr	Gly	Ile	Phe	Thr	Ala	Glu	Met	Thr	
				755					760					765	
TTC	AAG	ATC	ATT	GCC	CTC	GAC	CCC	TAC	TAC	TAC	TTC	CAA	CAG	GGC	2340
Phe	Lys	Ile	Ile	Ala	Leu	Asp	Pro	Tyr	Tyr	Tyr	Phe	Gln	Gln	Gly	
				770					775					780	
TGG	AAC	ATC	TTC	GAC	AGC	ATC	ATC	GTC	ATC	CTT	AGC	CTC	ATG	GAG	2385
Trp	Asn	Ile	Phe	Asp	Ser	Ile	Ile	Val	Ile	Leu	Ser	Leu	Met	Glu	
				785					790					795	
CTG	GGC	CTG	TCC	CGC	ATG	AGC	AAC	TTG	TCG	GTG	CTG	CGC	TCC	TTC	2430
Leu	Gly	Leu	Ser	Arg	Met	Ser	Asn	Leu	Ser	Val	Leu	Arg	Ser	Phe	
				800					805					810	
CGC	CTG	CTG	CGG	GTC	TTC	AAG	CTG	GCC	AAA	TCA	TGG	CCC	ACC	CTG	2475
Arg	Leu	Leu	Arg	Val	Phe	Lys	Leu	Ala	Lys	Ser	Trp	Pro	Thr	Leu	
				815					820					825	

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AAC ACA CTC ATC AAG ATC ATC GGG AAC TCA GTG GGG GCA CTG GGG	2520
Asn Thr Leu Ile Lys Ile Ile Gly Asn Ser Val Gly Ala Leu Gly	
830 835 840	
AAC CTG ACA CTG GTG CTA GCC ATC ATC GTG TTC ATC TTT GCT GTG	2565
Asn Leu Thr Leu Val Leu Ala Ile Ile Val Phe Ile Phe Ala Val	
845 850 855	
GTG GGC ATG CAG CTC TTT GGC AAG AAC TAC TCG GAG CTG AGG GAC	2610
Val Gly Met Gln Leu Phe Gly Lys Asn Tyr Ser Glu Leu Arg Asp	
860 865 870	
AGC GAC TCA GGC CTG CTG CCT CGC TGG CAC ATG ATG GAC TTC TTT	2655
Ser Asp Ser Gly Leu Leu Pro Arg Trp His Met Met Asp Phe Phe	
875 880 885	
CAT GCC TTC CTA ATC ATC TTC CGC ATC CTC TGT GGA GAG TGG ATC	2700
His Ala Phe Leu Ile Ile Phe Arg Ile Leu Cys Gly Glu Trp Ile	
890 895 900	
GAG ACC ATG TGG GAC TGC ATG GAG GTG TCG GGG CAG TCA TTA TGC	2745
Glu Thr Met Trp Asp Cys Met Glu Val Ser Gly Gln Ser Leu Cys	
905 910 915	
CTG CTG GTC TTC TTG CTT GTT ATG GTC ATT GGC AAC CTT GTG GTC	2790
Leu Leu Val Phe Leu Leu Val Met Val Ile Gly Asn Leu Val Val	
920 925 930	
CTG AAT CTC TTC CTG GCC TTG CTG CTC AGC TCC TTC AGT GCA GAC	2835
Leu Asn Leu Phe Leu Ala Leu Leu Leu Ser Ser Phe Ser Ala Asp	
935 940 945	
AAC CTC ACA GCC CCT GAT GAG GAC AGA GAG ATG AAC AAC CTC CAG	2880
Asn Leu Thr Ala Pro Asp Glu Asp Arg Glu Met Asn Asn Leu Gln	
950 955 960	
CTG GCC CTG GCC CGC ATC CAG AGG GGC CTG CGC TTT GTC AAG CGG	2925
Leu Ala Leu Ala Arg Ile Gln Arg Gly Leu Arg Phe Val Lys Arg	
965 970 975	
ACC ACC TGG GAT TTC TGC TGT GGT CTC CTG CGG CAC CGG CCT CAG	2970
Thr Thr Trp Asp Phe Cys Cys Gly Leu Leu Arg His Arg Pro Gln	
980 985 990	
AAG CCC GCA GCC CTT GCC GCC CAG GGC CAG CTG CCC AGC TGC ATT	3015
Lys Pro Ala Ala Leu Ala Ala Gln Gly Gln Leu Pro Ser Cys Ile	
995 1000 1005	
GCC ACC CCC TAC TCC CCG CCA CCC CCA GAG ACG GAG AAG GTG CCT	3060
Ala Thr Pro Tyr Ser Pro Pro Pro Pro Glu Thr Glu Lys Val Pro	
1010 1015 1020	
CCC ACC CGC AAG GAA ACA CAG TTT GAG GAA GGC GAG CAA CCA GGC	3105
Pro Thr Arg Lys Glu Thr Gln Phe Glu Glu Gly Glu Gln Pro Gly	
1025 1030 1035	
CAG GGC ACC CCC GGG GAT CCA GAC GCC GTG TGT GTG CCC ATC GCT	3150
Gln Gly Thr Pro Gly Asp Pro Glu Pro Val Cys Val Pro Ile Ala	
1040 1045 1050	
GTG GCC GAG TCA GAC ACA GAT GAC CAA GAA GAG GAT GAG GAG AAC	3195
Val Ala Glu Ser Asp Thr Asp Asp Gln Glu Glu Asp Glu Glu Asn	
1055 1060 1065	
AGC CTG GGC ACG GAG GAG GAG TCC AGC AAG CAG CAG GAA TCC CAG	3240
Ser Leu Gly Thr Glu Glu Glu Ser Ser Lys Gln Gln Glu Ser Gln	
1070 1075 1080	

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CCT	GTG	TCC	GGC	TGG	CCC	AGA	GGC	CCT	CCG	GAT	TCC	AGG	ACC	TGG	3285
Pro	Val	Ser	Gly	Trp	Pro	Arg	Gly	Pro	Pro	Asp	Ser	Arg	Thr	Trp	
				1085					1090					1095	
AGC	CAG	GTG	TCA	GCG	ACT	GCC	TCC	TCT	GAG	GCC	GAG	GCC	AGT	GCA	3330
Ser	Gln	Val	Ser	Ala	Thr	Ala	Ser	Ser	Glu	Ala	Glu	Ala	Ser	Ala	
				1100					1105					1110	
TCT	CAG	GCC	GAC	TGG	CGG	CAG	CAG	TGG	AAA	GCG	GAA	CCC	CAG	GCC	3375
Ser	Gln	Ala	Asp	Trp	Arg	Gln	Gln	Trp	Lys	Ala	Glu	Pro	Gln	Ala	
				1115					1120					1125	
CCA	GGG	TGC	GGT	GAG	ACC	CCA	GAG	GAC	AGT	TGC	TCC	GAG	GGC	AGC	3420
Pro	Gly	Cys	Gly	Glu	Thr	Pro	Glu	Asp	Ser	Cys	Ser	Glu	Gly	Ser	
				1130					1135					1140	
ACA	GCA	GAC	ATG	ACC	AAC	ACC	GCT	GAG	CTC	CTG	GAG	CAG	ATC	CCT	3465
Thr	Ala	Asp	Met	Thr	Asn	Thr	Ala	Glu	Leu	Leu	Glu	Gln	Ile	Pro	
				1145					1150					1155	
GAC	CTC	GGC	CAG	GAT	GTC	AAG	GAC	CCA	GAG	GAC	TGC	TTC	ACT	GAA	3510
Asp	Leu	Gly	Gln	Asp	Val	Lys	Asp	Pro	Glu	Asp	Cys	Phe	Thr	Glu	
				1160					1165					1170	
GGC	TGT	GTC	CGG	CGC	TGT	CCC	TGC	TGT	GCG	GTG	GAC	ACC	ACA	CAG	3555
Gly	Cys	Val	Arg	Arg	Cys	Pro	Cys	Cys	Ala	Val	Asp	Thr	Thr	Gln	
				1175					1180					1185	
GCC	CCA	GGG	AAG	GTC	TGG	TGG	CGG	TTG	CGC	AAG	ACC	TGC	TAC	CAC	3600
Ala	Pro	Gly	Lys	Val	Trp	Trp	Arg	Leu	Arg	Lys	Thr	Cys	Tyr	His	
				1190					1195					1200	
ATC	GTG	GAG	CAC	AGC	TGG	TTC	GAG	ACA	TTC	ATC	ATC	TTC	ATG	ATC	3645
Ile	Val	Glu	His	Ser	Trp	Phe	Glu	Thr	Phe	Ile	Ile	Phe	Met	Ile	
				1205					1210					1215	
CTA	CTC	AGC	AGT	GGA	GCG	CTG	GCC	TTC	GAG	GAC	ATC	TAC	CTA	GAG	3690
Leu	Leu	Ser	Ser	Gly	Ala	Leu	Ala	Phe	Glu	Asp	Ile	Tyr	Leu	Glu	
				1220					1225					1230	
GAG	CGG	AAG	ACC	ATC	AAG	GTT	CTG	CTT	GAG	TAT	GCC	GAC	AAG	ATG	3735
Glu	Arg	Lys	Thr	Ile	Lys	Val	Leu	Leu	Glu	Tyr	Ala	Asp	Lys	Met	
				1235					1240					1245	
TTC	ACA	TAT	GTC	TTC	GTG	CTG	GAG	ATG	CTG	CTC	AAG	TGG	GTG	GCC	3780
Phe	Thr	Tyr	Val	Phe	Val	Leu	Glu	Met	Leu	Leu	Lys	Trp	Val	Ala	
				1250					1255					1260	
TAC	GGC	TTC	AAG	AAG	TAC	TTC	ACC	AAT	GCC	TGG	TGC	TGG	CTC	GAC	3825
Tyr	Gly	Phe	Lys	Lys	Tyr	Phe	Thr	Asn	Ala	Trp	Cys	Trp	Leu	Asp	
				1265					1270					1275	
TTC	CTC	ATC	GTA	GAC	GTC	TCT	CTG	GTC	AGC	CTG	GTG	GCC	AAC	ACC	3870
Phe	Leu	Ile	Val	Asp	Val	Ser	Leu	Val	Ser	Leu	Val	Ala	Asn	Thr	
				1280					1285					1290	
CTG	GGC	TTT	GCC	GAG	ATG	GGC	CCC	ATC	AAG	TCA	CTG	CGG	ACG	CTG	3915
Leu	Gly	Phe	Ala	Glu	Met	Gly	Pro	Ile	Lys	Ser	Leu	Arg	Thr	Leu	
				1295					1300					1305	
CGT	GCA	CTC	CGT	CCT	CTG	AGA	GCT	CTG	TCA	CGA	TTT	GAG	GGC	ATG	3960
Arg	Ala	Leu	Arg	Pro	Leu	Arg	Ala	Leu	Ser	Arg	Phe	Glu	Gly	Met	
				1310					1315					1320	
AGG	GTG	GTG	GTC	AAT	GCC	CTG	GTG	GGC	GCC	ATC	CCG	TCC	ATC	ATG	4005
Arg	Val	Val	Val	Asn	Ala	Leu	Val	Gly	Ala	Ile	Pro	Ser	Ile	Met	
				1325					1330					1335	



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AAC GTC CTC CTC	GTC TGC CTC ATC TTC	TGG CTC ATC TTC AGC ATC	4050
Asn Val Leu Leu	Val Cys Leu Ile Phe	Trp Leu Ile Phe Ser Ile	
	1340	1345	1350
ATG GGC GTG AAC	CTC TTT GCG GGG AAG	TTT GGG AGG TGC ATC AAC	4095
Met Gly Val Asn	Leu Phe Ala Gly Lys	Phe Gly Arg Cys Ile Asn	
	1355	1360	1365
CAG ACA GAG GGA	GAC TTG CCT TTG AAC	TAC ACC ATC GTG AAC AAC	4140
Gln Thr Glu Gly	Asp Leu Pro Leu Asn	Tyr Thr Ile Val Asn Asn	
	1370	1375	1380
AAG AGC CAG TGT	GAG TCC TTG AAC TTG	ACC GGA GAA TTG TAC TGG	4185
Lys Ser Gln Cys	Glu Ser Leu Asn Leu	Thr Gly Glu Leu Tyr Trp	
	1385	1390	1395
ACC AAG GTG AAA	GTC AAC TTT GAC AAC	GTG GGG GCC GGG TAC CTG	4230
Thr Lys Val Lys	Val Asn Phe Asp Asn	Val Gly Ala Gly Tyr Leu	
	1400	1405	1410
GCC CTT CTG CAG	GTG GCA ACA TTT AAA	GGC TGG ATG GAC ATT ATG	4275
Ala Leu Leu Gln	Val Ala Thr Phe Lys	Gly Trp Met Asp Ile Met	
	1415	1420	1425
TAT GCA GCT GTG	GAC TCC AGG GGG TAT	GAA GAG CAG CCT CAG TGG	4320
Tyr Ala Ala Val	Asp Ser Arg Gly Tyr	Glu Glu Gln Pro Gln Trp	
	1430	1435	1440
GAA TAC AAC CTC	TAC ATG TAC ATC TAT	TTT GTC ATT TTC ATC ATC	4365
Glu Tyr Asn Leu	Tyr Met Tyr Ile Tyr	Phe Val Ile Phe Ile Ile	
	1445	1450	1455
TTT GGG TCT TTC	TTC ACC CTG AAC CTC	TTT ATT GGT GTC ATC ATT	4410
Phe Gly Ser Phe	Phe Thr Leu Asn Leu	Phe Ile Gly Val Ile Ile	
	1460	1465	1470
GAC AAC TTC AAC	CAA CAG AAG AAA AAG	TTA GGG GGC CAG GAC ATC	4455
Asp Asn Phe Asn	Gln Gln Lys Lys Lys	Leu Gly Gly Gln Asp Ile	
	1475	1480	1485
TTC ATG ACA GAG	GAG CAG AAG AAG TAC	TAC AAT GCC ATG AAG AAG	4500
Phe Met Thr Glu	Glu Gln Lys Lys Tyr	Tyr Asn Ala Met Lys Lys	
	1490	1495	1500
CTG GGC TCC AAG	AAG CCC CAG AAG CCC	ATC CCA CGG CCC CTG AAC	4545
Leu Gly Ser Lys	Lys Pro Gln Lys Pro	Ile Pro Arg Pro Leu Asn	
	1505	1510	1515
AAG TAC CAG GGC	TTC ATA TTC GAC ATT	GTG ACC AAG CAG GCC TTT	4590
Lys Tyr Gln Gly	Phe Ile Phe Asp Ile	Val Thr Lys Gln Ala Phe	
	1520	1525	1530
GAC GTC ACC ATC	ATG TTT CTG ATC TGC	TTG AAT ATG GTG ACC ATG	4635
Asp Val Thr Ile	Met Phe Leu Ile Cys	Leu Asn Met Val Thr Met	
	1535	1540	1545
ATG GTG GAG ACA	GAT GAC CAA AGT CCT	GAG AAA ATC AAC ATC TTG	4680
Met Val Glu Thr	Asp Asp Gln Ser Pro	Glu Lys Ile Asn Ile Leu	
	1550	1555	1560
GCC AAG ATC AAC	CTG CTC TTT GTG GCC	ATC TTC ACA GGC GAG TGT	4725
Ala Lys Ile Asn	Leu Leu Phe Val Ala	Ile Phe Thr Gly Glu Cys	
	1565	1570	1575
ATT GTC AAG CTG	GCT GCC CTG CGC CAC	TAC TAC TTC ACC AAC AGC	4770
Ile Val Lys Leu	Ala Ala Leu Arg His	Tyr Tyr Phe Thr Asn Ser	
	1580	1585	1590

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TGG AAT ATC TTC GAC TTC GTG GTT GTC ATC CTC TCC ATC GTG GGC	4815
Trp Asn Ile Phe Asp Phe Val Val Val Ile Leu Ser Ile Val Gly	
1595 1600 1605	
ACT GTG CTC TCG GAC ATC ATC CAG AAG TAC TTC TTC TCC CCG ACG	4860
Thr Val Leu Ser Asp Ile Ile Gln Lys Tyr Phe Phe Ser Pro Thr	
1610 1615 1620	
CTC TTC CGA GTC ATC CGC CTG GCC CGA ATA GGC CGC ATC CTC AGA	4905
Leu Phe Arg Val Ile Arg Leu Ala Arg Ile Gly Arg Ile Leu Arg	
1625 1630 1635	
CTG ATC CGA GGG GCC AAG GGG ATC CGC ACG CTG CTC TTT GCC CTC	4950
Leu Ile Arg Gly Ala Lys Gly Ile Arg Thr Leu Leu Phe Ala Leu	
1640 1645 1650	
ATG ATG TCC CTG CCT GCC CTC TTC AAC ATC GGC CTG CTG CTC TTC	4995
Met Met Ser Leu Pro Ala Leu Phe Asn Ile Gly Leu Leu Leu Phe	
1655 1660 1665	
CTC GTC ATG TTC ATC TAC TCC ATC TTT GGC ATG GCC AAC TTC GCT	5040
Leu Val Met Phe Ile Tyr Ser Ile Phe Gly Met Ala Asn Phe Ala	
1670 1675 1680	
TAT GTC AAG TGG GAG GCT GGC ATC GAC GAC ATG TTC AAC TTC CAG	5085
Tyr Val Lys Trp Glu Ala Gly Ile Asp Asp Met Phe Asn Phe Gln	
1685 1690 1695	
ACC TTC GCC AAC AGC ATG CTG TGC CTC TTC CAG ATC ACC ACG TCG	5130
Thr Phe Ala Asn Ser Met Leu Cys Leu Phe Gln Ile Thr Thr Ser	
1700 1705 1710	
GCC GGC TGG GAT GGC CTC CTC AGC CCC ATC CTC AAC ACT GGG CCG	5175
Ala Gly Trp Asp Gly Leu Leu Ser Pro Ile Leu Asn Thr Gly Pro	
1715 1720 1725	
CCC TAC TGC GAC CCC ACT CTG CCC AAC AGC AAT GGC TCT CGG GGG	5220
Pro Tyr Cys Asp Pro Thr Leu Pro Asn Ser Asn Gly Ser Arg Gly	
1730 1735 1740	
GAC TGC GGG AGC CCA GCC GTG GGC ATC CTC TTC TTC ACC ACC TAC	5265
Asp Cys Gly Ser Pro Ala Val Gly Ile Leu Phe Phe Thr Thr Tyr	
1745 1750 1755	
ATC ATC ATC TCC TTC CTC ATC GTG GTC AAC ATG TAC ATT GCC ATC	5310
Ile Ile Ile Ser Phe Leu Ile Val Val Asn Met Tyr Ile Ala Ile	
1760 1765 1770	
ATC CTG GAG AAC TTC AGC GTG GCC ACG GAG GAG AGC ACC GAG CCC	5355
Ile Leu Glu Asn Phe Ser Val Ala Thr Glu Glu Ser Thr Glu Pro	
1775 1780 1785	
CTG AGT GAG GAC GAC TTC GAT ATG TTC TAT GAG ATC TGG GAG AAA	5400
Leu Ser Glu Asp Asp Phe Asp Met Phe Tyr Glu Ile Trp Glu Lys	
1790 1795 1800	
TTT GAC CCA GAG GCC ACT CAG TTT ATT GAG TAT TCG GTC CTG TCT	5445
Phe Asp Pro Glu Ala Thr Gln Phe Ile Glu Tyr Ser Val Leu Ser	
1805 1810 1815	
GAC TTT GCC GAC GCC CTG TCT GAG CCA CTC CGT ATC GCC AAG CCC	5490
Asp Phe Ala Asp Ala Leu Ser Glu Pro Leu Ile Arg Ala Lys Pro	
1820 1825 1830	
AAC CAG ATA AGC CTC ATC AAC ATG GAC CTG CCC ATG GTG AGT GGG	5535
Asn Gln Ile Ser Leu Ile Asn Met Asp Leu Pro Met Val Ser Gly	
1835 1840 1845	

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GAC	CGC	ATC	CAT	TGC	ATG	GAC	ATT	CTC	TTT	GCC	TTC	ACC	AAA	AGG	5580
Asp	Arg	Ile	His	Cys	Met	Asp	Ile	Leu	Phe	Ala	Phe	Thr	Lys	Arg	
				1850					1855					1860	
GTC	CTG	GGG	GAG	TCT	GGG	GAG	ATG	GAC	GCC	CTG	AAG	ATC	CAG	ATG	5625
Val	Leu	Gly	Glu	Ser	Gly	Glu	Met	Asp	Ala	Leu	Lys	Ile	Gln	Met	
				1865					1870					1875	
GAG	GAG	AAG	TTC	ATG	GCA	GCC	AAC	CCA	TCC	AAG	ATC	TCC	TAC	GAG	5670
Glu	Glu	Lys	Phe	Met	Ala	Ala	Asn	Pro	Ser	Lys	Ile	Ser	Tyr	Glu	
				1880					1885					1890	
CCC	ATC	ACC	ACC	ACA	CTC	CGG	CGC	AAG	CAC	GAA	GAG	GTG	TCG	GCC	5715
Pro	Ile	Thr	Thr	Thr	Leu	Arg	Arg	Lys	His	Glu	Glu	Val	Ser	Ala	
				1895					1900					1905	
ATG	GTT	ATC	CAG	AGA	GCC	TTC	CGC	AGG	CAC	CTG	CTG	CAA	CGC	TCT	5760
Met	Val	Ile	Gln	Arg	Ala	Phe	Arg	Arg	His	Leu	Leu	Gln	Arg	Ser	
				1910					1915					1920	
TTG	AAG	CAT	GCC	TCC	TTC	CTC	TTC	CGT	CAG	CAG	GCG	GGC	AGC	GGC	5805
Leu	Lys	His	Ala	Ser	Phe	Leu	Phe	Arg	Gln	Gln	Ala	Gly	Ser	Gly	
				1925					1930					1935	
CTC	TCC	GAA	GAG	GAT	GCC	CCT	GAG	CGA	GAG	GGC	CTC	ATC	GCC	TAC	5850
Leu	Ser	Glu	Glu	Asp	Ala	Pro	Glu	Arg	Glu	Gly	Leu	Ile	Ala	Tyr	
				1940					1945					1950	
GTG	ATG	AGT	GAG	AAC	TTC	TCC	CGA	CCC	CTT	GGC	CCA	CCC	TCC	AGC	5895
Val	Met	Ser	Glu	Asn	Phe	Ser	Arg	Pro	Leu	Gly	Pro	Pro	Ser	Ser	
				1955					1960					1965	
TCC	TCC	ATC	TCC	TCC	ACT	TCC	TTC	CCA	CCC	TCC	TAT	GAC	AGT	GTC	5940
Ser	Ser	Ile	Ser	Ser	Thr	Ser	Phe	Pro	Pro	Ser	Tyr	Asp	Ser	Val	
				1970					1975					1980	
ACT	AGA	GCC	ACC	AGC	GAT	AAC	CTC	CAG	GTG	CGG	GGG	TCT	GAC	TAC	5985
Thr	Arg	Ala	Thr	Ser	Asp	Asn	Leu	Gln	Val	Arg	Gly	Ser	Asp	Tyr	
				1985					1990					1995	
AGC	CAC	AGT	GAA	GAT	CTC	GCC	GAC	TTC	CCC	CCT	TCT	CCG	GAC	AGG	6030
Ser	His	Ser	Glu	Asp	Leu	Ala	Asp	Phe	Pro	Pro	Ser	Pro	Asp	Arg	
				2000					2005					2010	
GAC	CGT	GAG	TCC	ATC	GTG										
Asp	Arg	Glu	Ser	Ile	Val										
				2015											

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2016 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Ala	Asn	Phe	Leu	Leu	Pro	Arg	Gly	Thr	Ser	Ser	Phe	Arg	Arg	
1				5					10					15	
Phe	Thr	Arg	Glu	Ser	Leu	Ala	Ala	Ile	Glu	Lys	Arg	Met	Ala	Glu	
				20					25					30	
Lys	Gln	Ala	Arg	Gly	Ser	Thr	Thr	Leu	Gln	Glu	Ser	Arg	Glu	Gly	
				35					40					45	
Leu	Pro	Glu	Glu	Glu	Ala	Pro	Arg	Pro	Gln	Leu	Asp	Leu	Gln	Ala	
				50					55					60	

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Ser	Lys	Lys	Leu	Pro	Asp	Leu	Tyr	Gly	Asn	Pro	Pro	Gln	Glu	Leu	65	70	75
Ile	Gly	Glu	Pro	Leu	Glu	Asp	Leu	Asp	Pro	Phe	Tyr	Ser	Thr	Gln	80	85	90
Lys	Thr	Phe	Ile	Val	Leu	Asn	Lys	Gly	Lys	Thr	Ile	Phe	Arg	Phe	95	100	105
Ser	Ala	Thr	Asn	Ala	Leu	Tyr	Val	Leu	Ser	Pro	Phe	His	Pro	Val	110	115	120
Arg	Arg	Ala	Ala	Val	Lys	Ile	Leu	Val	His	Ser	Leu	Phe	Asn	Met	125	130	135
Leu	Ile	Met	Cys	Thr	Ile	Leu	Thr	Asn	Cys	Val	Phe	Met	Ala	Gln	140	145	150
His	Asp	Pro	Pro	Pro	Trp	Thr	Lys	Tyr	Val	Glu	Tyr	Thr	Phe	Thr	155	160	165
Ala	Ile	Tyr	Thr	Phe	Glu	Ser	Leu	Val	Lys	Ile	Leu	Ala	Arg	Ala	170	175	180
Phe	Cys	Leu	His	Ala	Phe	Thr	Phe	Leu	Arg	Asp	Pro	Trp	Asn	Trp	185	190	195
Leu	Asp	Phe	Ser	Val	Ile	Ile	Met	Ala	Tyr	Thr	Thr	Glu	Phe	Val	200	205	210
Asp	Leu	Gly	Asn	Val	Ser	Ala	Leu	Arg	Thr	Phe	Arg	Val	Leu	Arg	215	220	225
Ala	Leu	Lys	Thr	Ile	Ser	Val	Ile	Ser	Gly	Leu	Lys	Thr	Ile	Val	230	235	240
Gly	Ala	Leu	Ile	Gln	Ser	Val	Lys	Lys	Leu	Ala	Asp	Val	Met	Val	245	250	255
Leu	Thr	Val	Phe	Cys	Leu	Ser	Val	Phe	Ala	Leu	Ile	Gly	Leu	Gln	260	265	270
Leu	Phe	Met	Gly	Asn	Leu	Arg	His	Lys	Cys	Val	Arg	Asn	Phe	Thr	275	280	285
Ala	Leu	Asn	Gly	Thr	Asn	Gly	Ser	Val	Glu	Ala	Asp	Gly	Leu	Val	290	295	300
Trp	Glu	Ser	Leu	Asp	Leu	Tyr	Leu	Ser	Asp	Pro	Glu	Asn	Tyr	Leu	305	310	315
Leu	Lys	Asn	Gly	Thr	Ser	Asp	Val	Leu	Leu	Cys	Gly	Asn	Ser	Ser	320	325	330
Asp	Ala	Gly	Thr	Cys	Pro	Glu	Gly	Tyr	Arg	Cys	Leu	Lys	Ala	Gly	335	340	345
Glu	Asn	Pro	Asp	His	Gly	Tyr	Thr	Ser	Phe	Asp	Ser	Phe	Ala	Trp	350	355	360
Ala	Phe	Leu	Ala	Leu	Phe	Arg	Leu	Met	Thr	Gln	Asp	Cys	Trp	Glu	365	370	375
Arg	Leu	Tyr	Gln	Gln	Thr	Leu	Arg	Ser	Ala	Gly	Lys	Ile	Tyr	Met	380	385	390

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Ile Phe Phe Met	Leu Val Ile Phe Leu Gly Ser Phe Tyr Leu Val	395	400	405
Asn Leu Ile Leu	Ala Val Val Ala Met Ala Tyr Glu Glu Gln Asn	410	415	420
Gln Ala Thr Ile	Ala Glu Thr Glu Glu Lys Glu Lys Arg Phe Gln	425	430	435
Glu Ala Met Glu	Met Leu Lys Lys Glu His Glu Ala Leu Thr Ile	440	445	450
Arg Gly Val Asp	Thr Val Ser Arg Ser Ser Leu Glu Met Ser Pro	455	460	465
Leu Ala Pro Val	Asn Ser His Glu Arg Arg Ser Lys Arg Arg Lys	470	475	480
Arg Met Ser Ser	Gly Thr Glu Glu Cys Gly Glu Asp Arg Leu Pro	485	490	495
Lys Ser Asp Ser	Glu Asp Gly Pro Arg Ala Met Asn His Leu Ser	500	505	510
Leu Thr Arg Gly	Leu Ser Arg Thr Ser Met Lys Pro Arg Ser Ser	515	520	525
Arg Gly Ser Ile	Phe Thr Phe Arg Arg Arg Asp Leu Gly Ser Glu	530	535	540
Ala Asp Phe Ala	Asp Asp Glu Asn Ser Thr Ala Arg Glu Ser Glu	545	550	555
Ser His His Thr	Ser Leu Leu Val Pro Trp Pro Leu Arg Arg Thr	560	565	570
Ser Ala Gln Gly	Gln Pro Ser Pro Gly Thr Ser Ala Pro Gly His	575	580	585
Ala Leu His Gly	Lys Lys Asn Ser Thr Val Asp Cys Asn Gly Val	590	595	600
Val Ser Leu Leu	Gly Ala Gly Asp Pro Glu Ala Thr Ser Pro Gly	605	610	615
Ser His Leu Leu	Arg Pro Val Met Leu Glu His Pro Pro Asp Thr	620	625	630
Thr Thr Pro Ser	Glu Glu Pro Gly Gly Pro Gln Met Leu Thr Ser	635	640	645
Gln Ala Pro Cys	Val Asp Gly Phe Glu Glu Pro Gly Ala Arg Gln	650	655	660
Arg Ala Leu Ser	Ala Val Ser Val Leu Thr Ser Ala Leu Glu Glu	665	670	675
Leu Glu Glu Ser	Arg His Lys Cys Pro Pro Cys Trp Asn Arg Leu	680	685	690
Ala Gln Arg Tyr	Leu Ile Trp Glu Cys Cys Pro Leu Trp Met Ser	695	700	705
Ile Lys Gln Gly	Val Lys Leu Val Val Met Asp Pro Phe Thr Asp	710	715	720

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Leu Thr Ile Thr	Met Cys Ile Val	Leu Asn Thr	Leu Phe Met	Ala
	725	730		735
Leu Glu His Tyr	Asn Met Thr Ser	Glu Phe Glu	Glu Met Leu	Gln
	740	745		750
Val Gly Asn Leu	Val Phe Thr Gly	Ile Phe Thr	Ala Glu Met	Thr
	755	760		765
Phe Lys Ile Ile	Ala Leu Asp Pro	Tyr Tyr Tyr	Phe Gln Gln	Gly
	770	775		780
Trp Asn Ile Phe	Asp Ser Ile Ile	Val Ile Leu	Ser Leu Met	Glu
	785	790		795
Leu Gly Leu Ser	Arg Met Ser Asn	Leu Ser Val	Leu Arg Ser	Phe
	800	805		810
Arg Leu Leu Arg	Val Phe Lys Leu	Ala Lys Ser	Trp Pro Thr	Leu
	815	820		825
Asn Thr Leu Ile	Lys Ile Ile Gly	Asn Ser Val	Gly Ala Leu	Gly
	830	835		840
Asn Leu Thr Leu	Val Leu Ala Ile	Ile Val Phe	Ile Phe Ala	Val
	845	850		855
Val Gly Met Gln	Leu Phe Gly Lys	Asn Tyr Ser	Glu Leu Arg	Asp
	860	865		870
Ser Asp Ser Gly	Leu Leu Pro Arg	Trp His Met	Met Asp Phe	Phe
	875	880		885
His Ala Phe Leu	Ile Ile Phe Arg	Ile Leu Cys	Gly Glu Trp	Ile
	890	895		900
Glu Thr Met Trp	Asp Cys Met Glu	Val Ser Gly	Gln Ser Leu	Cys
	905	910		915
Leu Leu Val Phe	Leu Leu Val Met	Val Ile Gly	Asn Leu Val	Val
	920	925		930
Leu Asn Leu Phe	Leu Ala Leu Leu	Leu Ser Ser	Phe Ser Ala	Asp
	935	940		945
Asn Leu Thr Ala	Pro Asp Glu Asp	Arg Glu Met	Asn Asn Leu	Gln
	950	955		960
Leu Ala Leu Ala	Arg Ile Gln Arg	Gly Leu Arg	Phe Val Lys	Arg
	965	970		975
Thr Thr Trp Asp	Phe Cys Cys Gly	Leu Leu Arg	His Arg Pro	Gln
	980	985		990
Lys Pro Ala Ala	Leu Ala Ala Gln	Gly Gln Leu	Pro Ser Cys	Ile
	995	1000		1005
Ala Thr Pro Tyr	Ser Pro Pro Pro	Pro Glu Thr	Glu Lys Val	Pro
	1010	1015		1020
Pro Thr Arg Lys	Glu Thr Gln Phe	Glu Glu Gly	Gln Gln Pro	Gly
	1025	1030		1035
Gln Gly Thr Pro	Gly Asp Pro Glu	Pro Val Cys	Val Pro Ile	Ala
	1040	1045		1050

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Val	Ala	Glu	Ser	Asp	Thr	Asp	Asp	Gln	Glu	Glu	Asp	Glu	Glu	Asn	
				1055					1060					1065	
Ser	Leu	Gly	Thr	Glu	Glu	Glu	Ser	Ser	Lys	Gln	Gln	Glu	Ser	Gln	
				1070					1075					1080	
Pro	Val	Ser	Gly	Trp	Pro	Arg	Gly	Pro	Pro	Asp	Ser	Arg	Thr	Trp	
				1085					1090					1095	
Ser	Gln	Val	Ser	Ala	Thr	Ala	Ser	Ser	Glu	Ala	Glu	Ala	Ser	Ala	
				1100					1105					1110	
Ser	Gln	Ala	Asp	Trp	Arg	Gln	Gln	Trp	Lys	Ala	Glu	Pro	Gln	Ala	
				1115					1120					1125	
Pro	Gly	Cys	Gly	Glu	Thr	Pro	Glu	Asp	Ser	Cys	Ser	Glu	Gly	Ser	
				1130					1135					1140	
Thr	Ala	Asp	Met	Thr	Asn	Thr	Ala	Glu	Leu	Leu	Glu	Gln	Ile	Pro	
				1145					1150					1155	
Asp	Leu	Gly	Gln	Asp	Val	Lys	Asp	Pro	Glu	Asp	Cys	Phe	Thr	Glu	
				1160					1165					1170	
Gly	Cys	Val	Arg	Arg	Cys	Pro	Cys	Cys	Ala	Val	Asp	Thr	Thr	Gln	
				1175					1180					1185	
Ala	Pro	Gly	Lys	Val	Trp	Trp	Arg	Leu	Arg	Lys	Thr	Cys	Tyr	His	
				1190					1195					1200	
Ile	Val	Glu	His	Ser	Trp	Phe	Glu	Thr	Phe	Ile	Ile	Phe	Met	Ile	
				1205					1210					1215	
Leu	Leu	Ser	Ser	Gly	Ala	Leu	Ala	Phe	Glu	Asp	Ile	Tyr	Leu	Glu	
				1220					1225					1230	
Glu	Arg	Lys	Thr	Ile	Lys	Val	Leu	Leu	Glu	Tyr	Ala	Asp	Lys	Met	
				1235					1240					1245	
Phe	Thr	Tyr	Val	Phe	Val	Leu	Glu	Met	Leu	Leu	Lys	Trp	Val	Ala	
				1250					1255					1260	
Tyr	Gly	Phe	Lys	Lys	Tyr	Phe	Thr	Asn	Ala	Trp	Cys	Trp	Leu	Asp	
				1265					1270					1275	
Phe	Leu	Ile	Val	Asp	Val	Ser	Leu	Val	Ser	Leu	Val	Ala	Asn	Thr	
				1280					1285					1290	
Leu	Gly	Phe	Ala	Glu	Met	Gly	Pro	Ile	Lys	Ser	Leu	Arg	Thr	Leu	
				1295					1300					1305	
Arg	Ala	Leu	Arg	Pro	Leu	Arg	Ala	Leu	Ser	Arg	Phe	Glu	Gly	Met	
				1310					1315					1320	
Arg	Val	Val	Val	Asn	Ala	Leu	Val	Gly	Ala	Ile	Pro	Ser	Ile	Met	
				1325					1330					1335	
Asn	Val	Leu	Leu	Val	Cys	Leu	Ile	Phe	Trp	Leu	Ile	Phe	Ser	Ile	
				1340					1345					1350	
Met	Gly	Val	Asn	Leu	Phe	Ala	Gly	Lys	Phe	Gly	Arg	Cys	Ile	Asn	
				1355					1360					1365	
Gln	Thr	Glu	Gly	Asp	Leu	Pro	Leu	Asn	Tyr	Thr	Ile	Val	Asn	Asn	
				1370					1375					1380	

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Lys Ser Gln Cys	Glu Ser Leu Asn Leu	Thr Gly Glu Leu Tyr	Trp
	1385	1390	1395
Thr Lys Val Lys	Val Asn Phe Asp Asn	Val Gly Ala Gly Tyr	Leu
	1400	1405	1410
Ala Leu Leu Gln	Val Ala Thr Phe Lys	Gly Trp Met Asp Ile	Met
	1415	1420	1425
Tyr Ala Ala Val	Asp Ser Arg Gly Tyr	Glu Glu Gln Pro Gln	Trp
	1430	1435	1440
Glu Tyr Asn Leu	Tyr Met Tyr Ile Tyr	Phe Val Ile Phe Ile	Ile
	1445	1450	1455
Phe Gly Ser Phe	Phe Thr Leu Asn Leu	Phe Ile Gly Val Ile	Ile
	1460	1465	1470
Asp Asn Phe Asn	Gln Gln Lys Lys Lys	Leu Gly Gly Gln Asp	Ile
	1475	1480	1485
Phe Met Thr Glu	Glu Gln Lys Lys Tyr	Tyr Asn Ala Met Lys	Lys
	1490	1495	1500
Leu Gly Ser Lys	Lys Pro Gln Lys Pro	Ile Pro Arg Pro Leu	Asn
	1505	1510	1515
Lys Tyr Gln Gly	Phe Ile Phe Asp Ile	Val Thr Lys Gln Ala	Phe
	1520	1525	1530
Asp Val Thr Ile	Met Phe Leu Ile Cys	Leu Asn Met Val Thr	Met
	1535	1540	1545
Met Val Glu Thr	Asp Asp Gln Ser Pro	Glu Lys Ile Asn Ile	Leu
	1550	1555	1560
Ala Lys Ile Asn	Leu Leu Phe Val Ala	Ile Phe Thr Gly Glu	Cys
	1565	1570	1575
Ile Val Lys Leu	Ala Ala Leu Arg His	Tyr Tyr Phe Thr Asn	Ser
	1580	1585	1590
Trp Asn Ile Phe	Asp Phe Val Val Val	Ile Leu Ser Ile Val	Gly
	1595	1600	1605
Thr Val Leu Ser	Asp Ile Ile Gln Lys	Tyr Phe Phe Ser Pro	Thr
	1610	1615	1620
Leu Phe Arg Val	Ile Arg Leu Ala Arg	Ile Gly Arg Ile Leu	Arg
	1625	1630	1635
Leu Ile Arg Gly	Ala Lys Gly Ile Arg	Thr Leu Leu Phe Ala	Leu
	1640	1645	1650
Met Met Ser Leu	Pro Ala Leu Phe Asn	Ile Gly Leu Leu Leu	Phe
	1655	1660	1665
Leu Val Met Phe	Ile Tyr Ser Ile Phe	Gly Met Ala Asn Phe	Ala
	1670	1675	1680
Tyr Val Lys Trp	Glu Ala Gly Ile Asp	Asp Met Phe Asn Phe	Gln
	1685	1690	1695
Thr Phe Ala Asn	Ser Met Leu Cys Leu	Phe Gln Ile Thr Thr	Ser
	1700	1705	1710



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Ala Gly Trp Asp	Gly Leu Leu Ser Pro	Ile Leu Asn Thr Gly Pro
	1715	1720 1725
Pro Tyr Cys Asp	Pro Thr Leu Pro Asn	Ser Asn Gly Ser Arg Gly
	1730	1735 1740
Asp Cys Gly Ser	Pro Ala Val Gly Ile	Leu Phe Phe Thr Thr Tyr
	1745	1750 1755
Ile Ile Ile Ser	Phe Leu Ile Val Val	Asn Met Tyr Ile Ala Ile
	1760	1765 1770
Ile Leu Glu Asn	Phe Ser Val Ala Thr	Glu Glu Ser Thr Glu Pro
	1775	1780 1785
Leu Ser Glu Asp	Asp Phe Asp Met Phe	Tyr Glu Ile Trp Glu Lys
	1790	1795 1800
Phe Asp Pro Glu	Ala Thr Gln Phe Ile	Glu Tyr Ser Val Leu Ser
	1805	1810 1815
Asp Phe Ala Asp	Ala Leu Ser Glu Pro	Leu Ile Arg Ala Lys Pro
	1820	1825 1830
Asn Gln Ile Ser	Leu Ile Asn Met Asp	Leu Pro Met Val Ser Gly
	1835	1840 1845
Asp Arg Ile His	Cys Met Asp Ile Leu	Phe Ala Phe Thr Lys Arg
	1850	1855 1860
Val Leu Gly Glu	Ser Gly Glu Met Asp	Ala Leu Lys Ile Gln Met
	1865	1870 1875
Glu Glu Lys Phe	Met Ala Ala Asn Pro	Ser Lys Ile Ser Tyr Glu
	1880	1885 1890
Pro Ile Thr Thr	Thr Leu Arg Arg Lys	His Glu Glu Val Ser Ala
	1895	1900 1905
Met Val Ile Gln	Arg Ala Phe Arg Arg	His Leu Leu Gln Arg Ser
	1910	1915 1920
Leu Lys His Ala	Ser Phe Leu Phe Arg	Gln Gln Ala Gly Ser Gly
	1925	1930 1935
Leu Ser Glu Glu	Asp Ala Pro Glu Arg	Glu Gly Leu Ile Ala Tyr
	1940	1945 1950
Val Met Ser Glu	Asn Phe Ser Arg Pro	Leu Gly Pro Pro Ser Ser
	1955	1960 1965
Ser Ser Ile Ser	Ser Thr Ser Phe Pro	Pro Ser Tyr Asp Ser Val
	1970	1975 1980
Thr Arg Ala Thr	Ser Asp Asn Leu Gln	Val Arg Gly Ser Asp Tyr
	1985	1990 1995
Ser His Ser Glu	Asp Leu Ala Asp Phe	Pro Pro Ser Pro Asp Arg
	2000	2005 2010
Asp Arg Glu Ser	Ile Val	
	2015	

- (2) INFORMATION FOR SEQ ID NO:3:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 24 bases

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- (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGCAAACCT TCCTATTACC TCGG 24

- (2) INFORMATION FOR SEQ ID NO:4:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 24 bases  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CACGATGGAC TCACGGTCCC TGTC 24

- (2) INFORMATION FOR SEQ ID NO:5:  
 (I) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 3069 bases  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATG GGG AAG GGG GTT GGA CGT GAT AAG TAT GAG CCT GCA GCT GTT	45
Met Gly Lys Gly Val Gly Arg Asp Lys Tyr Glu Pro Ala Ala Val	
1 5 10 15	
TCA GAA CAA GGT GAT AAA AAG GGC AAA AAG GGC AAA AAA GAC AGG	90
Ser Glu Gln Glu Asp Lys Lys Glu Lys Lys Glu Lys Lys Asp Arg	
20 25 30	
GAC ATG GAT GAA CTG AAG AAA GAA GTT TCT ATG GAT GAT CAT AAA	135
Asp Met Asp Glu Leu Lys Lys Glu Val Ser Met Asp Asp His Lys	
35 40 45	
CTT AGC CTT GAT GAA CTT CAT CGT AAA TAT GGA ACA GAC TTG AGC	180
Leu Ser Leu Asp Glu Leu His Arg Lys Tyr Gly Thr Asp Leu Ser	
50 55 60	
CGG GGA TTA ACA TCT GCT CGT GCA GCT GAG ATC CTG GCG CGA GAT	225
Arg Gly Leu Thr Ser Ala Arg Ala Ala Glu Ile Leu Ala Arg Asp	
65 70 75	
GGT CCC AAC GCC CTC ACT CCC CCT CCC ACT ACT CCT GAA TGG ATC	270
Gly Pro Asn Ala Leu Thr Pro Pro Pro Thr Thr Pro Glu Trp Ile	
80 85 90	
AAG TTT TGT CGG CAG CTC TTT GGG GGG TTC TCA ATG TTA CTG TGG	315
Lys Phe Cys Arg Gln Leu Phe Gly Gly Phe Ser Met Leu Leu Trp	
95 100 105	
ATT GGA GCG ATT CTT TGT TTC TTG GCT TAT AGC ATC CAA GCT GCT	360
Ile Gly Ala Ile Leu Cys Phe Leu Ala Tyr Ser Ile Gln Ala Ala	
110 115 120	
ACA GAA GAG GAA CCT CAA AAC GAT AAT CTG TAC CTG GGT GTG GTG	405
Thr Glu Glu Glu Pro Gln Asn Asp Asn Leu Tyr Leu Gly Val Val	
125 130 135	
CTA TCA GCC GTT GTA ATC ATA ACT GGT TGC TTC TCC TAC TAT CAA	450
Leu Ser Ala Val Val Ile Ile Thr Gly Cys Phe Ser Tyr Tyr Gln	
140 145 150	

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GAA GCT AAA AGT TCA AAG ATC ATG GAA TCC TTC AAA AAC ATG GTC	495
Glu Ala Lys Ser Ser Lys Ile Met Glu Ser Phe Lys Asn Met Val	
155 160 165	
CCT CAG CAA GCC CTT GTG ATT CGA AAT GGT GAG AAA ATG AGC ATA	540
Pro Gln Gln Ala Leu Val Ile Arg Asn Gly Glu Lys Met Ser Ile	
170 175 180	
AAT GCG GAG GAA GTT GTG GTT GGG GAT CTG GTG GAA GTA AAA GGA	585
Asn Ala Glu Glu Val Val Val Gly Asp Lue Val Glu Val Lys Gly	
185 190 195	
GGA GAC CGA ATT CCT GCT GAC CTC AGA ATC ATA TCT GCA AAT GGC	630
Gly Asp Arg Ile Pro Ala Asp Leu Arg Ile Ile Ser Ala Asn Gly	
200 205 210	
TGC AAG GTG GAT AAC TCC TCG CTC ACT GGT GAA TCA GAA CCC CAG	675
Cys Lys Val Asp Asn Ser Ser Leu Thr Gly Glu Ser Glu Pro Gln	
215 220 225	
ACT AGG TCT CCA GAT TTC ACA AAT GAA AAC CCC CTG GAG ACG AGG	720
Thr Arg Ser Pro Asp Phe Thr Asn Glu Asn Pro Leu Glu Thr Arg	
230 235 240	
AAC ATT GCC TTC TTT TCA ACA AAT TGT GTT GAA GGC ACC GCA CGT	765
Asn Ile Ala Phe Phe Ser Thr Asn Cys Val Glu Gly Thr Ala Arg	
245 250 255	
GGT ATT GTT GTC TAC ACT GGG GAT CGC ACT GTG ATG GGA AGA ATT	810
Gly Ile Val Val Tyr Thr Gly Asp Arg Thr Val Met Gly Arg Ile	
260 265 270	
GCC ACA CTT GCT TCT GGG CTG GAA GGA GGC CAG ACC CCC ATT GCT	855
Ala Thr Leu Ala Ser Gly Leu Glu Gly Gly Gln Thr Pro Ile Ala	
275 280 285	
GCA GAA ATT GAA CAT TTT ATC CAC ATC ATC ACG GGT GTG GCT GTG	900
Ala Glu Ile Glu His Phe Ile His Ile Ile Thr Gly Val Ala Val	
290 295 300	
TTC CTG GGT GTG TCT TTC TTC ATC CTT TCT CTC ATC CTT GAG TAC	945
Phe Leu Gly Val Ser Phe Phe Ile Leu Ser Leu Ile Leu Glu Tyr	
305 310 315	
ACC TGG CTT GAG GCT GTC ATC TTC CTC ATC GGT ATC ATC GTA GCC	990
Thr Trp Leu Glu Ala Val Ile Phe Leu Ile Gly Ile Ile Val Ala	
320 325 330	
AAT GTG CCG GAA GGT TTG CTG GCC ACT GTC ACG GTC TGT CTG ACA	1035
Asn Val Pro Glu Gly Leu Leu Ala Thr Val Thr Val Cys Leu Thr	
335 340 345	
CTT ACT GCC AAA CGC ATG GCA AGG AAA AAC TGC TTA GTG AAG AAC	1080
Leu Thr Ala Lys Arg Met Ala Arg Lys Asn Cys Leu Val Lys Asn	
350 355 360	
TTA GAA GCT GTG GAG ACC TTG GGG TCC ACG TCC ACC ATC TGC TCT	1125
Leu Glu Ala Val Glu Thr Leu Gly Ser Thr Ser Thr Ile Cys Ser	
365 370 375	
GAT AAA ACT GGA ACT CTG ACT CAG AAC CGG ATG ACA GTG GCC CAC	1170
Asp Lys Thr Gly Thr Leu Thr Gln Asn Arg Met Thr Val Ala His	
380 385 390	
ATG TGG TTT GAC AAT CAA ATC CAT GAA GCT GAT ACG ACA GAG AAT	1215
Met Trp Phe Asp Asn Gln Ile His Glu Ala Asp Thr Thr Glu Asn	
395 400 405	

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CAG AGT GGT GTC TCT TTT GAC AAG ACT TCA GCT ACC TGG CTT GCT	1260
Gln Ser Gly Val Ser Phe Asp Lys Thr Ser Ala Thr Trp Leu Ala	
410 415 420	
CTG TCC AGA ATT GCA GGT CTT TGT AAC AGG GCA GTG TTT CAG GCT	1305
Leu Ser Arg Ile Ala Gly Leu Cys Asn Arg Ala Val Phe Gln Ala	
425 430 435	
AAC CAG GAA AAC CTA CCT ATT CTT AAG CGG GCA GTT GCA GGA GAT	1350
Asn Gln Glu Asn Leu Pro Ile Leu Lys Arg Ala Val Ala Gly Asp	
440 445 450	
GCC TCT GAG TCA GCA CTC TTA AAG TGC ATA GAG CTG TGC TGT GGT	1395
Ala Ser Glu Ser Ala Leu Leu Lys Cys Ile Glu Leu Cys Cys Gly	
455 460 465	
TTC GTG AAG GAG ATG AGA GAA AGA TAC GCC AAA ATC GTC GAG ATA	1440
Ser Val Lys Glu Met Arg Glu Arg Tyr Ala Lys Ile Val Glu Ile	
470 475 480	
CCC TTC AAC TCC ACC AAC AAG TAC CAG TTG TCT ATT CAT AAG AAC	1485
Pro Phe Asn Ser Thr Asn Lys Tyr Gln Leu Ser Ile His Lys Asn	
485 490 495	
CCC AAC ACA TCG GAG CCC CAA CAC CTG TTG GTG ATG AAG GGC GCC	1520
Pro Asn Thr Ser Glu Pro Gln His Leu Leu Val Met Lys Gly Ala	
500 505 510	
CCA GAA AGG ATC CTA GAC CGT TGC AGC TCT ATC CTC CTC CAC GGC	1565
Pro Glu Arg Ile Leu Asp Arg Cys Ser Ser Ile Leu Leu His Gly	
515 520 525	
AAG GAG CAG CCC CTG GAT GAG GAG CTG AAA GAC GCC TTT CAG AAC	1620
Lys Glu Gln Pro Leu Asp Glu Glu Leu Lys Asp Ala Phe Gln Asn	
530 535 540	
GCC TAT TTG GAG CTG GGG GGC CTC GGA GAA CGA GTC CTA GGT TTC	1665
Ala Tyr Leu Glu Leu Gly Gly Leu Gly Glu Arg Val Leu Gly Phe	
545 550 555	
TGC CAC CTC TTT CTG CCA GAT GAA CAG TTT CCT GAA GGG TTC CAG	1710
Cys His Leu Phe Leu Pro Asp Glu Gln Phe Pro Glu Gly Phe Gln	
560 565 570	
TTT GAC ACT GAC GAT GTG AAT TTC CCT ATC GAT AAT CTG TGC TTC	1755
Phe Asp Thr Asp Asp Val Asn Phe Pro Ile Asp Asn Leu Cys Phe	
575 580 585	
GTT GGG CTC ATC TCC ATG ATT GAC CCT CCA CGG GCG GCC GTT CCT	1800
Val Gly Leu Ile Ser Met Ile Asp Pro Pro Arg Ala Ala Val Pro	
590 595 600	
GAT GCC GTG GGC AAA TGT CGA AGT GCT GGA ATT AAG GTC ATC ATG	1845
Asp Ala Val Gly Lys Cys Arg Ser Aal Gly Ile Lys Val Ile Met	
605 610 615	
GTC ACA GGA GAC CAT CCA ATC ACA GCT AAA GCT ATT GCC AAA GGT	1890
Val Thr Gly Asp His Pro Ile Thr Ala Lys Ala Ile Ala Lys Gly	
620 625 630	
GTG GGC ATC ATC TCA GAA GGC ATG GAG ACC GTG GAA GAC ATT GCT	1935
Val Gly Ile Ile Ser Glu Gly Asn Glu Thr Val Glu Asp Ile Ala	
635 640 645	
GCC CGC CTC AAC ATC CCA GTC AGC CAG GTG AAC CCC AGG GAT GCC	1980
Ala Arg Leu Asn Ile Pro Val Ser Gln Val Asn Pro Arg Asp Ala	
650 655 660	

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AAG GCC TGC GTA GTA CAC GGC AGT GAT CTA AAG GAC ATG ACC TCC	2025
Lys Ala Cys Val Val His Gly Ser Asp Leu Lys Asp Met Thr Ser	
665 670 675	
GAG CAG CTG GAT GAC ATT TTG AAG TAC CAC ACT GAG ATA GTG TTT	2070
Glu Gln Leu Asp Asp Ile Leu Lys Tyr His Thr Glu Ile Val Phe	
680 685 690	
GCC AGG ACC TCC CCT CAG CAG AAG CTC ATC ATT GTG GAA GGC TGC	2115
Ala Arg Thr Ser Pro Gln Gln Lys Leu Ile Ile Val Glu Gly Cys	
695 700 705	
CAA AGA CAG GGT GCT ATC GTG GCT GTG ACT GGT GAC GGT GTG AAT	2160
Gln Arg Gln Gly Ala Ile Val Ala Val Thr Gly Asp Gly Val Asn	
710 715 720	
GAC TCT CCA GCT TTG AAG AAA GCA GAC ATT GGG GTT GCT ATG GGG	2205
Asp Ser Pro Ala Leu Lys Lys Ala Asp Ile Gly Val Ala Met Gly	
725 730 735	
ATT GCT GGC TCA GAT GTG TCC AAG CAA GCT GCT GAC ATG ATT CTT	2250
Ile Ala Gly Ser Asp Val Ser Lys Gln Ala Ala Asp Met Ile Leu	
740 745 750	
CTG GAT GAC AAC TTT GCC TCA ATT GTG ACT GGA GTA GAG GAA GGT	2295
Leu Asp Asp Asn Phe Ala Ser Ile Val Thr Gly Val Glu Glu Gly	
755 760 765	
CGT CTG ATC TTT GAT AAC TTG AAG AAA TCC ATT GCT TAT ACC TTA	2340
Arg Leu Ile Phe Asp Asn Leu Lys Lys Ser Ile Ala Tyr Thr Leu	
770 775 780	
ACC AGT AAC ATT CCC GAG ATC ACC CCG TTC CTG ATA TTT ATT ATT	2385
Thr Ser Asn Ile Pro Glu Ile Thr Pro Phe Leu Ile Phe Ile Ile	
785 790 795	
GCA AAC ATT CCA CTA CCA CTG GGC ACT GTC ACC ATC CTC TGC ATT	2430
Ala Asn Ile Pro Leu Pro Leu Gly Thr Val Thr Ile Leu Cys Ile	
800 805 810	
GAC TTG GGC ACT GAC ATG GTT CCT GCC ATC TCC CTG GCT TAT GAG	2475
Asp Leu Gly Thr Asp Met Val Pro Ala Ile Ser Leu Ala Tyr Glu	
815 820 825	
CAG GCT GAG AGT GAC ATC ATG AAG AGA CAG CCC AGA AAT CCC AAA	2520
Gln Ala Glu Ser Asp Ile Met Lys Arg Gln Pro Arg Asn Pro Lys	
830 835 840	
ACA GAC AAA CTT GTG AAT GAG CGG CTG ATC AGC ATG GCC TAT GGG	2565
Thr Asp Lys Leu Val Asn Glu Arg Leu Ile Ser Met Ala Tyr Gly	
845 850 855	
CAG ATT GGA ATG ATC CAG GCC CTG GGA GGC TTC TTT ACT TAC TTT	2610
Gln Ile Gly Met Ile Gln Ala Leu Gly Gly Phe Phe Thr Tyr Phe	
860 865 870	
GTG ATT CTG GCT GAG AAC GGC TTC CTC CCA ATT CAC CTG TTG GGC	2655
Val Ile Leu Ala Glu Asn Gly Phe Leu Pro Ile His Leu Leu Gly	
875 880 885	
CTC CGA GTG GAC TGG GAT GAC CGC TGG ATC AAC GAT GTG GAA GAC	2700
Leu Arg Val Asp Trp Asp Asp Arg Trp Ile Asn Asp Val Glu Asp	
890 895 900	
AGC TAC GGG CAG CAG TGG ACC TAT GAG CAG AGG AAA ATC GTG GAG	2745
Ser Tyr Gly Gln Gln Trp Thr Tyr Glu Gln Arg Lys Ile Val Glu	
905 910 915	

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TTC ACC TGC CAC ACA GCC TTC TTC GTC AGT ATC GTG GTG GTG CAG	2790
Phe Thr Cys His Thr Ala Phe Phe Val Ser Ile Val Val Val Gln	
920 925 930	
TGG GCC GAC TTG GTC ATC TGT AAG ACC AGG AGG AAT TCG GTC TTC	2835
Trp Ala Asp Leu Val Ile Cys Lys Thr Arg Arg Asn Ser Val Phe	
935 940 945	
CAG CAG GGG ATG AAG AAC AAG ATC TTG ATA TTT GGC CTC TTT GAA	2880
Gln Gln Gly Met Lys Asn Lys Ile Leu Ile Phe Gly Leu Phe Glu	
950 955 960	
GAG ACA GCC CTG GCT GCT TTC CTT TCC TAC TGC CCT GGA ATG GGT	2925
Glu Thr Ala Leu Ala Ala Phe Leu Ser Tyr Cys Pro Gly Met Gly	
965 970 975	
GTT GCT CTT AGG ATG TAT CCC CTC AAA CCT ACC TGG TGG TTC TGT	2970
Val Ala Leu Arg Met Tyr Pro Leu Lys Pro Thr Trp Trp Phe Cys	
980 985 990	
GCC TTC CCC TAC TCT CTT CTC ATC TTC GTA TAT GAC GAA GTC AGA	3015
Ala Phe Pro Tyr Ser Leu Leu Ile Phe Val Tyr Asp Glu Val Arg	
995 1000 1005	
AAA CTC ATC ATC AGG CGA CGC CCT GGC GGC TGG GTG GAG AAG GAA	3060
Lys Leu Ile Ile Arg Arg Arg Pro Gly Gly Trp Val Glu Lys Glu	
1010 1015 1020	
ACC TAC TAT	3069
Thr Tyr Tyr	

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH:1023 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Gly Lys Gly Val Gly Arg Asp Lys Tyr Glu Pro Ala Ala Val	1 5 10 15
Ser Glu Gln Glu Asp Lys Lys Glu Lys Lys Glu Lys Lys Asp Arg	20 25 30
Asp Met Asp Glu Leu Lys Lys Glu Val Ser Met Asp Asp His Lys	35 40 45
Leu Ser Leu Asp Glu Leu His Arg Lys Tyr Gly Thr Asp Leu Ser	50 55 60
Arg Gly Leu Thr Ser Ala Arg Ala Ala Glu Ile Leu Ala Arg Asp	65 70 75
Gly Pro Asn Ala Leu Thr Pro Pro Pro Thr Thr Pro Glu Trp Ile	80 85 90
Lys Phe Cys Arg Gln Leu Phe Gly Gly Phe Ser Met Leu Leu Trp	95 100 105
Ile Gly Ala Ile Leu Cys Phe Leu Ala Tyr Ser Ile Gln Ala Ala	110 115 120
Thr Glu Glu Glu Pro Gln Asn Asp Asn Leu Tyr Leu Gly Val Val	125 130 135

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Leu Ser Ala Val	Val Ile Ile Thr Gly Cys Phe Ser Tyr Tyr Gln	140	145	150
Glu Ala Lys Ser	Ser Lys Ile Met Glu Ser Phe Lys Asn Met Val	155	160	165
Pro Gln Gln Ala	Leu Val Ile Arg Asn Gly Glu Lys Met Ser Ile	170	175	180
Asn Ala Glu Glu	Val Val Val Gly Asp Lue Val Glu Val Lys Gly	185	190	195
Gly Asp Arg Ile	Pro Ala Asp Leu Arg Ile Ile Ser Ala Asn Gly	200	205	210
Cys Lys Val Asp	Asn Ser Ser Leu Thr Gly Glu Ser Glu Pro Gln	215	220	225
Thr Arg Ser Pro	Asp Phe Thr Asn Glu Asn Pro Leu Glu Thr Arg	230	235	240
Asn Ile Ala Phe	Phe Ser Thr Asn Cys Val Glu Gly Thr Ala Arg	245	250	255
Gly Ile Val Val	Tyr Thr Gly Asp Arg Thr Val Met Gly Arg Ile	260	265	270
Ala Thr Leu Ala	Ser Gly Leu Glu Gly Gly Gln Thr Pro Ile Ala	275	280	285
Ala Glu Ile Glu	His Phe Ile His Ile Ile Thr Gly Val Ala Val	290	295	300
Phe Leu Gly Val	Ser Phe Phe Ile Leu Ser Leu Ile Leu Glu Tyr	305	310	315
Thr Trp Leu Glu	Ala Val Ile Phe Leu Ile Gly Ile Ile Val Ala	320	325	330
Asn Val Pro Glu	Gly Leu Leu Ala Thr Val Thr Val Cys Leu Thr	335	340	345
Leu Thr Ala Lys	Arg Met Ala Arg Lys Asn Cys Leu Val Lys Asn	350	355	360
Leu Glu Ala Val	Glu Thr Leu Gly Ser Thr Ser Thr Ile Cys Ser	365	370	375
Asp Lys Thr Gly	Thr Leu Thr Gln Asn Arg Met Thr Val Ala His	380	385	390
Met Trp Phe Asp	Asn Gln Ile His Glu Ala Asp Thr Thr Glu Asn	395	400	405
Gln Ser Gly Val	Ser Phe Asp Lys Thr Ser Ala Thr Trp Leu Ala	410	415	420
Leu Ser Arg Ile	Ala Gly Leu Cys Asn Arg Ala Val Phe Gln Ala	425	430	435
Asn Gln Glu Asn	Leu Pro Ile Leu Lys Arg Ala Val Ala Gly Asp	440	445	450
Ala Ser Glu Ser	Ala Leu Leu Lys Cys Ile Glu Leu Cys Cys Gly	455	460	465

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Ser Val Lys Glu	Met Arg Glu Arg Tyr	Ala Lys Ile Val Glu Ile	470	475	480
Pro Phe Asn Ser	Thr Asn Lys Tyr Gln	Leu Ser Ile His Lys Asn	485	490	495
Pro Asn Thr Ser	Glu Pro Gln His Leu	Leu Val Met Lys Gly Ala	500	505	510
Pro Glu Arg Ile	Leu Asp Arg Cys Ser	Ser Ile Leu Leu His Gly	515	520	525
Lys Glu Gln Pro	Leu Asp Glu Glu Leu	Lys Asp Ala Phe Gln Asn	530	535	540
Ala Tyr Leu Glu	Leu Gly Gly Leu Gly	Glu Arg Val Leu Gly Phe	545	550	555
Cys His Leu Phe	Leu Pro Asp Glu Gln	Phe Pro Glu Gly Phe Gln	560	565	570
Phe Asp Thr Asp	Asp Val Asn Phe Pro	Ile Asp Asn Leu Cys Phe	575	580	585
Val Gly Leu Ile	Ser Met Ile Asp Pro	Pro Arg Ala Ala Val Pro	590	595	600
Asp Ala Val Gly	Lys Cys Arg Ser Aal	Gly Ile Lys Val Ile Met	605	610	615
Val Thr Gly Asp	His Pro Ile Thr Ala	Lys Ala Ile Ala Lys Gly	620	625	630
Val Gly Ile Ile	Ser Glu Gly Asn Glu	Thr Val Glu Asp Ile Ala	635	640	645
Ala Arg Leu Asn	Ile Pro Val Ser Gln	Val Asn Pro Arg Asp Ala	650	655	660
Lys Ala Cys Val	Val His Gly Ser Asp	Leu Lys Asp Met Thr Ser	665	670	675
Glu Glm Leu Asp	Asp Ile Leu Lys Tyr	His Thr Glu Ile Val Phe	680	685	690
Ala Arg Thr Ser	Pro Gln Gln Lys Leu	Ile Ile Val Glu Gly Cys	695	700	705
Gln Arg Gln Gly	Ala Ile Val Ala Val	Thr Gly Asp Gly Val Asn	710	715	720
Asp Ser Pro Ala	Leu Lys Lys Ala Asp	Ile Gly Val Ala Met Gly	725	730	735
Ile Ala Gly Ser	Asp Val Ser Lys Gln	Ala Ala Asp Met Ile Leu	740	745	750
Leu Asp Asp Asn	Phe Ala Ser Ile Val	Thr Gly Val Glu Glu Gly	755	760	765
Arg Leu Ile Phe	Asp Asn Leu Lys Lys	Ser Ile Ala Tyr Thr Leu	770	775	780
Thr Ser Asn Ile	Pro Glu Ile Thr Pro	Phe Leu Ile Phe Ile Ile	785	790	795



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Ala Asn Ile Pro Leu Pro Leu Gly Thr Val Thr Ile Leu Cys Ile  
800 805 810

Asp Leu Gly Thr Asp Met Val Pro Ala Ile Ser Leu Ala Tyr Glu  
815 820 825

Gln Ala Glu Ser Asp Ile Met Lys Arg Gln Pro Arg Asn Pro Lys  
830 835 840

Thr Asp Lys Leu Val Asn Glu Arg Leu Ile Ser Met Ala Tyr Gly  
845 850 855

Gln Ile Gly Met Ile Gln Ala Leu Gly Gly Phe Phe Thr Tyr Phe  
860 865 870

Val Ile Leu Ala Glu Asn Gly Phe Leu Pro Ile His Leu Leu Gly  
875 880 885

Leu Arg Val Asp Trp Asp Asp Arg Trp Ile Asn Asp Val Glu Asp  
890 895 900

Ser Tyr Gly Gln Gln Trp Thr Tyr Glu Gln Arg Lys Ile Val Glu  
905 910 915

Phe Thr Cys His Thr Ala Phe Phe Val Ser Ile Val Val Val Gln  
920 925 930

Trp Ala Asp Leu Val Ile Cys Lys Thr Arg Arg Asn Ser Val Phe  
935 940 945

Gln Gln Gly Met Lys Asn Lys Ile Leu Ile Phe Gly Leu Phe Glu  
950 955 960

Glu Thr Ala Leu Ala Ala Phe Leu Ser Tyr Cys Pro Gly Met Gly  
965 970 975

Val Ala Leu Arg Met Tyr Pro Leu Lys Pro Thr Trp Trp Phe Cys  
980 985 990

Ala Phe Pro Tyr Ser Leu Leu Ile Phe Val Tyr Asp Glu Val Arg  
995 1000 1005

Lys Leu Ile Ile Arg Arg Arg Pro Gly Gly Trp Val Glu Lys Glu  
1010 1015 1020

Thr Tyr Tyr

## (2) INFORMATION FOR SEQ ID NO:7:

## (I) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 909 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATG GCC CGC GGG AAA GCC AAG GAG GAG GGC AGC TGG AAG AAA TTC	45
Met Ala Arg Gly Lys Ala Lys Glu Glu Ser Trp Lys Lys Phe	15
1 5 10	
ATC TGG AAC TCA GAG AAG AAG GAG TTT CTG GGC AGG ACC GGT GGC	90
Ile Trp Asn Ser Glu Lys Lys Glu Phe Leu Gly Arg Thr Gly Gly	30
20 25 30	
AGT TGG TTT AAG ATC CTT CTA TTC TAC GTA ATA TTT TAT GGC TGC	135
Ser Trp Phe Lys Ile Leu Leu Phe Tyr Val Ile Phe Tyr Gly Cys	45
35 40 45	

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CTG GCT GGC ATC TTC ATC GGA ACC ATC CAA GTG ATG CTG CTC ACC	180
Leu Ala Gly Ile Phe Ile Gly Thr Ile Gln Val Met Leu Leu Thr	
50 55 60	
ATC AGT GAA TTT AAG CCC ACA TAT CAG GAC CGA GTG GCC CCG CCA	225
Ile Ser Glu Phe Lys Pro Thr Tyr Gln Asp Arg Val Ala Pro Pro	
65 70 75	
GGA TTA ACA CAG ATT CCT CAG ATC CAG AAG ACT GAA ATT TCC TTT	270
Gly Leu Thr Gln Ile Pro Gln Ile Gln Lys Thr Glu Ile Ser Phe	
80 85 90	
CGT CCT AAT GAT CCC AAG AGC TAT GAG GCA TAT GTA CTG AAC ATA	315
Arg Pro Asn Asp Pro Lys Ser Tyr Glu Ala Tyr Val Leu Asn Ile	
95 100 105	
GTT AGG TTC CTG GAA AAG TAC AAA GAT TCA GCC CAG AGG GAT GAC	360
Val Arg Phe Leu Glu Lys Tyr Lys Asp Ser Ala Gln Arg Asp Asp	
110 115 120	
ATG ATT TTT GAA GAT TGT GGC GAT GTG CCC AGT GAA CCG AAA GAA	405
Met Ile Phe Glu Asp Cys Gly Asp Val Pro Ser Glu Pro Lys Glu	
125 130 135	
CGA GGA GAC TTT AAT CAT GAA CGA GGA GAG CGA AAG GTC TGC AGA	450
Arg Gly Asp Phe Asn His Glu Arg Gly Glu Arg Lys Val Cys Arg	
140 145 150	
TTC AAG CTT GAA TGG CTG GGA AAT TGC TCT GGA TTA AAT GAT GAA	495
Phy Lys Leu Glu Trp Leu Gly Asn Cys Ser Gly Leu Asn Asp GAA	
155 160 165	
ACT TAT GGC TAC AAA GAG GGC AAA CCG TGC ATT ATT ATA AAG CTC	540
Thr Tyr Gly Tyr Lys Glu Gly Lys Pro Cys Ile Ile Ile Lys Leu	
170 175 180	
AAC CGA GTT CTA GGC TTC AAA CCT AAG CCT CCC AAG AAT GAG TCC	585
Asn Arg Val Leu Gly Phe Lys Pro Lys Pro Pro Lys Asn Glu Ser	
185 190 195	
TTG GAG ACT TAC CCA GTG ATG AAG TAT AAC CCA AAT GTC CTT CCC	630
Leu Glu Thr Tyr Pro Val Met Lys Tyr Asn Asn Pro Asn Val Leu Pro	
200 205 210	
GTT CAG TGC ACT GGC AAG CGA GAT GAA GAT AAG GAT AAA GTT GGA	675
Val Gln Cys Thr Gly Lys Arg Asp Glu Asp Lys Asp Lys Val Gly	
215 220 225	
AAT GTG GAG TAT TTT GGA CTG GGC AAC TCC CCT GGT TTT CCT CTG	720
Asn Val Glu Tyr Phe Gly Leu Gly Asn Ser Pro Gly Phe Pro Leu	
230 235 240	
CAG TAT TAT CCG TAC TAT GGC AAA CTC CTG CAG CCC AAA TAC CTG	765
Gln Tyr Tyr Pro Tyr Gly Lys Leu Leu Gln Pro Lys Tyr Leu	
245 250 255	
CAG CCC CTG CTG GCC GTA CAG TTC ACC AAT CTT ACC ATG GAC ACT	810
Gln Pro Leu Leu Ala Val Gln Phe Thr Asn Leu Thr Met Asp Thr	
260 265 270	
GAA ATT CGC ATA GAG TGT AAG GCG TAC GGT GAG AAC ATT GGG TAC	855
Glu Ile Arg Ile Glu Cys Lys Ala Tyr Gly Glu Asn Ile Gly Tyr	
275 280 285	
AGT GAG AAA GAC CGT TTT CAG GGA CGT TTT GAT GTA AAA ATT GAA	900
Ser Glu Lys Asp Arg Phe Gln Gly Arg Phe Asp Val Lys Ile Glu	
290 295 300	

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GTT AAG AGC 909  
Val Lys Ser

- (2) INFORMATION FOR SEQ ID NO:8:  
 (i) SEQUENCE CHARACTERISTICS:  
     (A) LENGTH:303 amino acids  
     (B) TYPE: amino acid  
     (C) STRANDEDNESS: single  
     (D) TOPOLOGY: unknown  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Ala	Arg	Gly	Lys	Ala	Lys	Glu	Glu	Gly	Ser	Trp	Lys	Lys	Phe	1	5	10	15
Ile	Trp	Asn	Ser	Glu	Lys	Lys	Glu	Phe	Leu	Gly	Arg	Thr	Gly	Gly	20	25	30	
Ser	Trp	Phe	Lys	Ile	Leu	Leu	Phe	Tyr	Val	Ile	Phe	Tyr	Gly	Cys	35	40	45	
Leu	Ala	Gly	Ile	Phe	Ile	Gly	Thr	Ile	Gln	Val	Met	Leu	Leu	Thr	50	55	60	
Ile	Ser	Glu	Phe	Lys	Pro	Thr	Tyr	Gln	Asp	Arg	Val	Ala	Pro	Pro	65	70	75	
Gly	Leu	Thr	Gln	Ile	Pro	Gln	Ile	Gln	Lys	Thr	Glu	Ile	Ser	Phe	80	85	90	
Arg	Pro	Asn	Asp	Pro	Lys	Ser	Tyr	Glu	Ala	Tyr	Val	Leu	Asn	Ile	95	100	105	
Val	Arg	Phe	Leu	Glu	Lys	Tyr	Lys	Asp	Ser	Ala	Gln	Arg	Asp	Asp	110	115	120	
Met	Ile	Phe	Glu	Asp	Cys	Gly	Asp	Val	Pro	Ser	Glu	Pro	Lys	Glu	125	130	135	
Arg	Gly	Asp	Phe	Asn	His	Glu	Arg	Gly	Glu	Arg	Lys	Val	Cys	Arg	140	145	150	
Phy	Lys	Leu	Glu	Trp	Leu	Gly	Asn	Cys	Ser	Gly	Leu	Asn	Asp	Glu	155	160	165	
Thr	Tyr	Gly	Tyr	Lys	Glu	Gly	Lys	Pro	Cys	Ile	Ile	Ile	Lys	Leu	170	175	180	
Asn	Arg	Val	Leu	Gly	Phe	Lys	Pro	Lys	Pro	Pro	Lys	Asn	Glu	Ser	185	190	195	
Leu	Glu	Thr	Tyr	Pro	Val	Met	Lys	Tyr	Asn	Pro	Asn	Val	Leu	Pro	200	205	210	
Val	Gln	Cys	Thr	Gly	Lys	Arg	Asp	Glu	Asp	Lys	Asp	Lys	Val	Gly	215	220	225	
Asn	Val	Glu	Tyr	Phe	Gly	Leu	Gly	Asn	Ser	Pro	Gly	Phe	Pro	Leu	230	235	240	
Gln	Tyr	Tyr	Pro	Tyr	Tyr	Gly	Lys	Leu	Leu	Gln	Pro	Lys	Tyr	Leu	245	250	255	
Gln	Pro	Leu	Leu	Ala	Val	Gln	Phe	Thr	Asn	Leu	Thr	Met	Asp	Thr	260	265	270	

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Glu Ile Arg Ile Glu Cys Lys Ala Tyr Gly Glu Asn Ile Gly Tyr  
275 280 285

Ser Glu Lys Asp Arg Phe Gln Gly Arg Phe Asp Val Lys Ile Glu  
290 295 300

Val Lys Ser

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGGGGAAGG GGGTTGGACG TGAT 24

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATAGTAGGTT TCCTTCTCCA CCCA 24

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGGCCCGCG GGAAAGCCAA GGAG 24

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCTCTTAACT TCAATTTTTC CATC 24

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## WHAT IS CLAIMED IS:

1. A delivery system for delivering a therapeutically effective amount of a predetermined genetic material to myocardial cells of a chosen location of a patient's heart, said genetic material being selected for the function of increasing the amplitude of the patient's cardiac signal so that it can be better sensed by an electrode, comprising:
  - 10 a supply of said genetic material;  
reservoir means for containing said genetic material; and  
delivery means for delivering said genetic material from said reservoir to said myocardial cells,  
15 thereby increasing the amplitude of the cardiac signal and improving the signal to noise ratio that can be sensed by a pacemaker.
2. The delivery system of claim 1, wherein said supply of genetic material comprises a bolus of ion channel protein genetic material selected for the function of increasing the amplitude of the cardiac signal.
3. The delivery system of claim 1, wherein said delivery means comprises a catheter with a distal end portion, and said reservoir means is located in said distal end portion.
4. The delivery system of claim 3, wherein said distal end portion comprises a hollow helical element forming an interior, and said reservoir means comprises said interior with said supply therein.
- 30 5. The delivery system of claim 1, wherein said delivery means comprises a catheter with a lumen for delivering said genetic material therethrough, said catheter having a distal tip communicating with said lumen for

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contacting said plurality of cells in the proximity of said electrode with said genetic material.

6. The delivery system of claim 5, wherein said distal tip is a hollow helical needle tip.

5           7. The delivery system of claim 5, wherein said catheter is a transvenous endocardial catheter.

8. The delivery system of claim 1, wherein said reservoir contains a supply of 0.1-10 ml of said genetic material.

10           9. The delivery system of claim 1, wherein said delivery means comprises a catheter with a distal portion and an end tip, and wherein said reservoir means is contained in said distal portion, and further comprising force means for forcing said genetic material from said  
15 reservoir means and out of said end tip.

10. The delivery system of claim 9, wherein said force means comprises a stylet.

11. The delivery system of claim 1, wherein said delivery system comprises a hollow helical screw-in element  
20 loaded with a bolus of said genetic material.

12. The delivery system of claim 11, wherein said element comprises ports for egress of said genetic material into said identified cardiac location when said element is screwed into said location, and further comprising soluble  
25 plugs in said ports to maintain them normally closed but which dissolve when said element is positioned within said patient's heart.

13. The delivery system of claim 1, wherein said predetermined genetic material is DNA or RNA, and imparts

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chronic change in ion channel expression in said cardiac cells.

14. The delivery system of claim 1, wherein said delivery means comprises a catheter with a distal end  
5 portion, and said reservoir means is located in said distal end portion.

15. The delivery system of claim 13, wherein said DNA or RNA encodes an ion channel protein.

16. The delivery system of claim 15, wherein said  
10 ion channel protein is a sodium channel protein.

17. The delivery system of claim 16, wherein said sodium channel protein is hH1.

18. The delivery system of claim 1, wherein said predetermined genetic material is protein, and imparts acute  
15 change in sodium channel expression in said cardiac cells.

19. The delivery system of claim 18, wherein said protein is an ion channel protein.

20. The delivery system of claim 19, wherein said ion channel protein is a sodium channel protein.

21. The delivery system of claim 20, wherein said  
20 sodium channel protein is hH1.

22. An implantable delivery system for delivering doses of a therapeutically effective amount of a predetermined genetic material to myocardial cells in a  
25 chosen location of a patient's heart, comprising:

a supply of genetic material of the class having the property of increasing the expression of ion channels in the myocardial cells to which it is delivered;

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a catheter, said catheter having a distal tip portion for engaging the cells of said chosen location and delivering thereto said genetic material;

reservoir means for holding said supply of genetic material and providing it to said distal tip portion of said catheter; and

delivery means for delivering a therapeutically effective amount of said genetic material from said reservoir means through said distal tip portion to said chosen location.

23. The system as described in claim 20, further comprising:

control means for controlling operation of said delivery means to deliver respective said doses.

24. The implantable delivery system of claim 23, wherein said control means comprises initiating means for initiating delivery of said genetic material, said initiating means comprising an external programmer.

25. The implantable delivery system of claim 23, wherein said control means comprises automatic means for automatically initiating delivery of said genetic material.

26. An implantable delivery system for delivering predetermined genetic material to cardiac cells adjacent to a pacing electrode positioned against the inner wall of a patient's heart, comprising:

a supply of genetic material of the class having the property of increasing the expression of ion channels in cardiac cells to which it is delivered;

a catheter, said catheter having a distal tip portion for engaging said cardiac cells and delivering thereto said genetic material;



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reservoir means for holding said supply of genetic material and providing it to said distal tip portion of said catheter; and

5 delivery means for delivering a therapeutically effective amount of said genetic material from said reservoir means through said distal tip portion to said cardiac cells.

27. The implantable delivery system of claim 26, wherein the distal end of said distal tip portion further  
10 comprises a pacing electrode.

28. The system as described in claim 26, further comprising:

control means for controlling operation of said delivery means to deliver respective said doses.

15 29. The implantable delivery system of claim 26, wherein said control means comprises initiating means for initiating delivery of said genetic material, said initiating means comprising an external programmer.

30. The implantable delivery system of claim 26,  
20 wherein said control means comprises automatic means for automatically initiating delivery of said genetic material.

31. An implantable system for pacing a patient's heart and for delivering a predetermined genetic material to cardiac cells adjacent to a pacing electrode positioned in  
25 said patient's heart, comprising:

a supply of genetic material of the class having the property of increasing the expression of ion channels in cardiac cells to which it is delivered;

a catheter, said catheter having proximal and  
30 distal ends, a lumen through at least a part thereof and connecting to said distal end, a pacing electrode positioned at said distal end for engaging said patient's heart wall,

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said electrode having a channel therethrough in communication with said lumen, and a conductor connecting said proximal end to said electrode,

5 a pulse generator connected electrically to said conductor at said catheter proximal end for delivering pace pulses to said electrode,

reservoir means for holding said supply of genetic material, and

10 delivery means for delivering said genetic material from said reservoir to said lumen, whereby said material passes through said lumen and said channel to said heart wall.

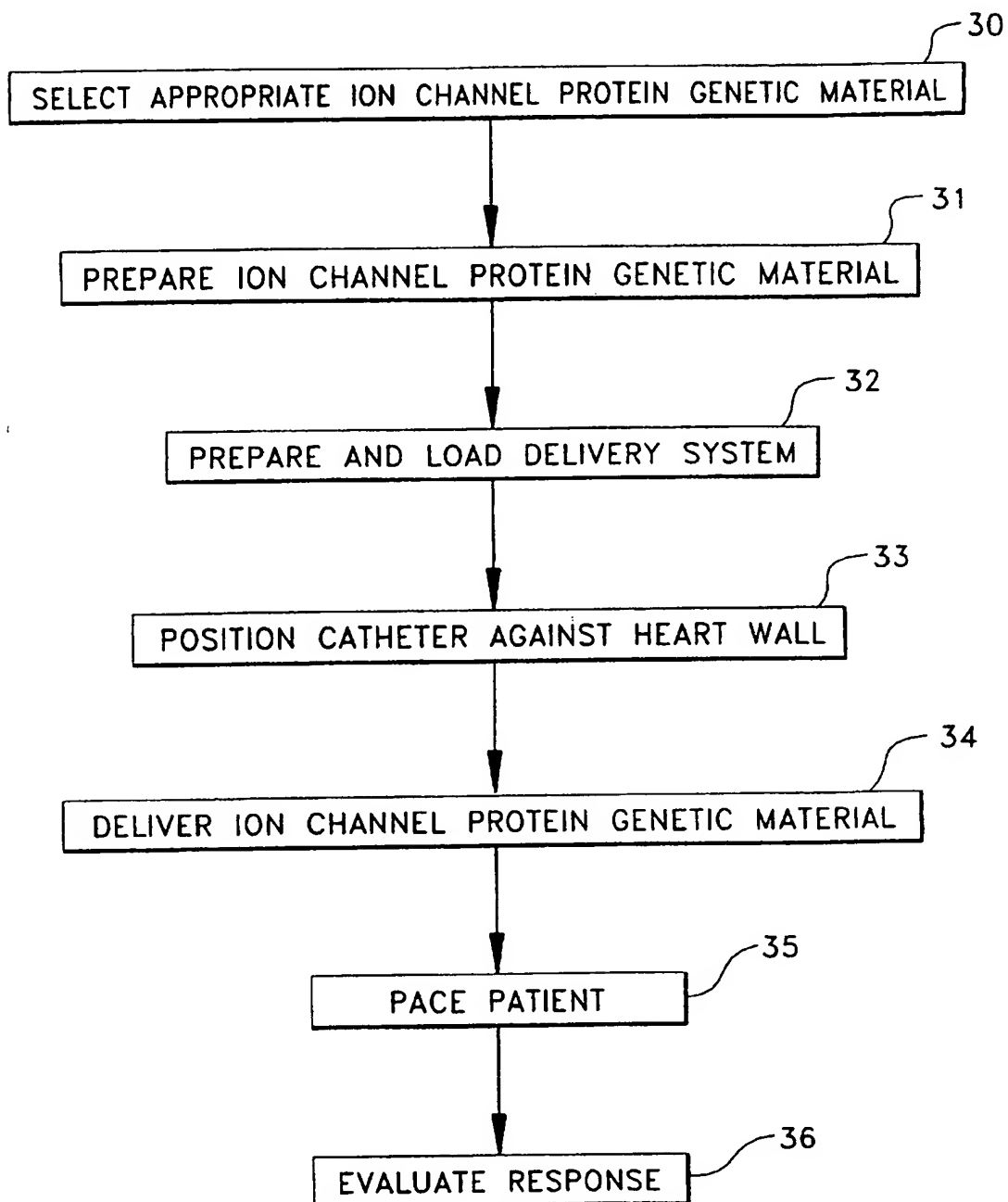
32. The implantable system of claim 31, wherein said reservoir is mounted in said pulse generator.

15 33. The implantable system of claim 31, wherein said delivery means is passive.

34. The implantable system of claim 31, wherein said delivery means comprises a pump.

20 35. The implantable system of claim 31, wherein said electrode is substantially concentric with respect to the catheter axis, and the channel passes through the center of said electrode.

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*FIG. 1*

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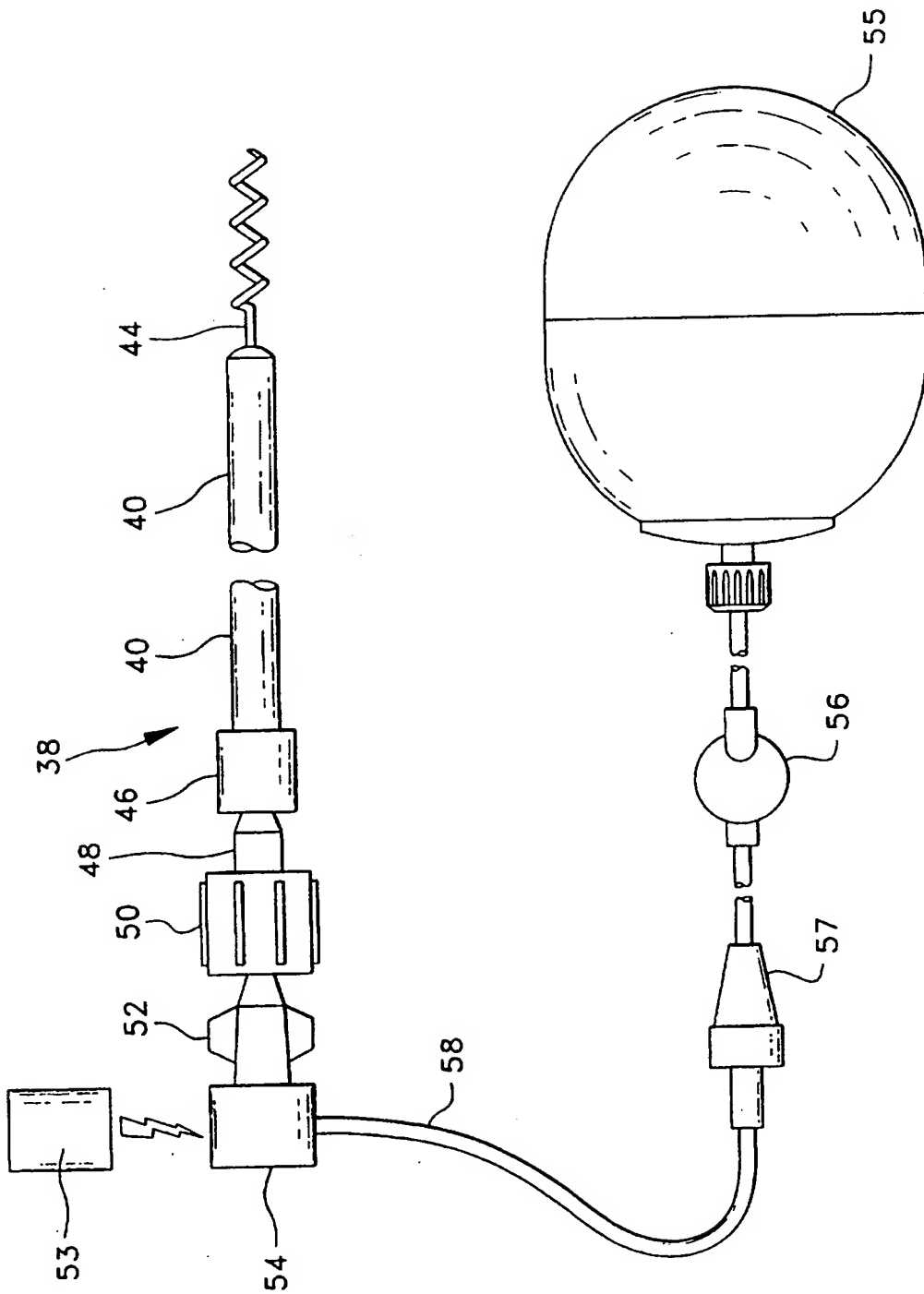


FIG. 2

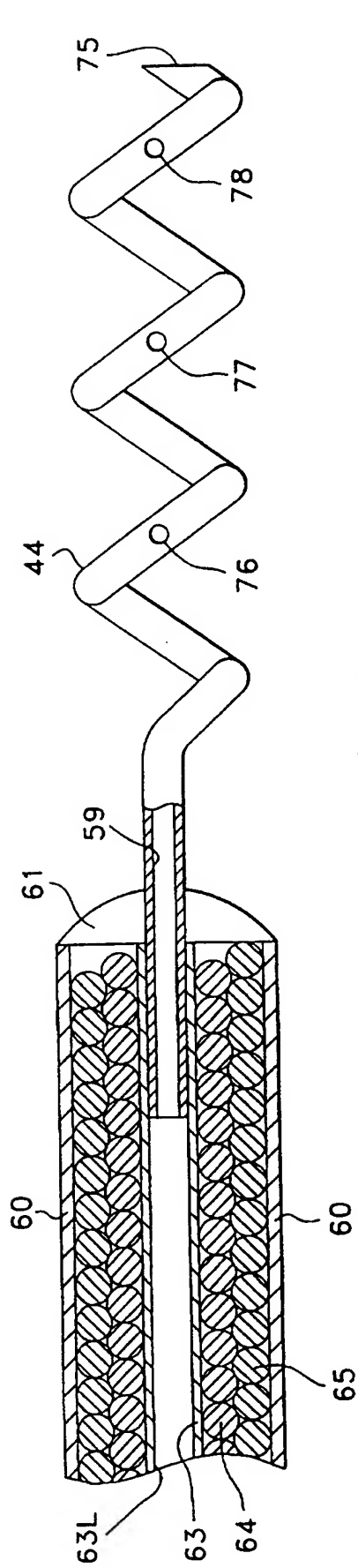


FIG. 3

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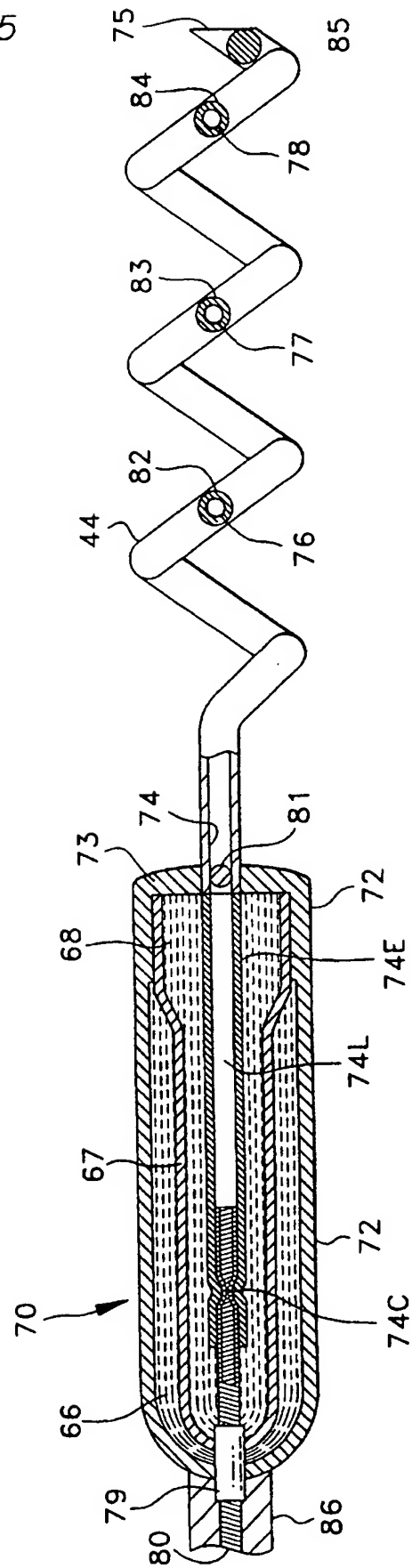


FIG. 4

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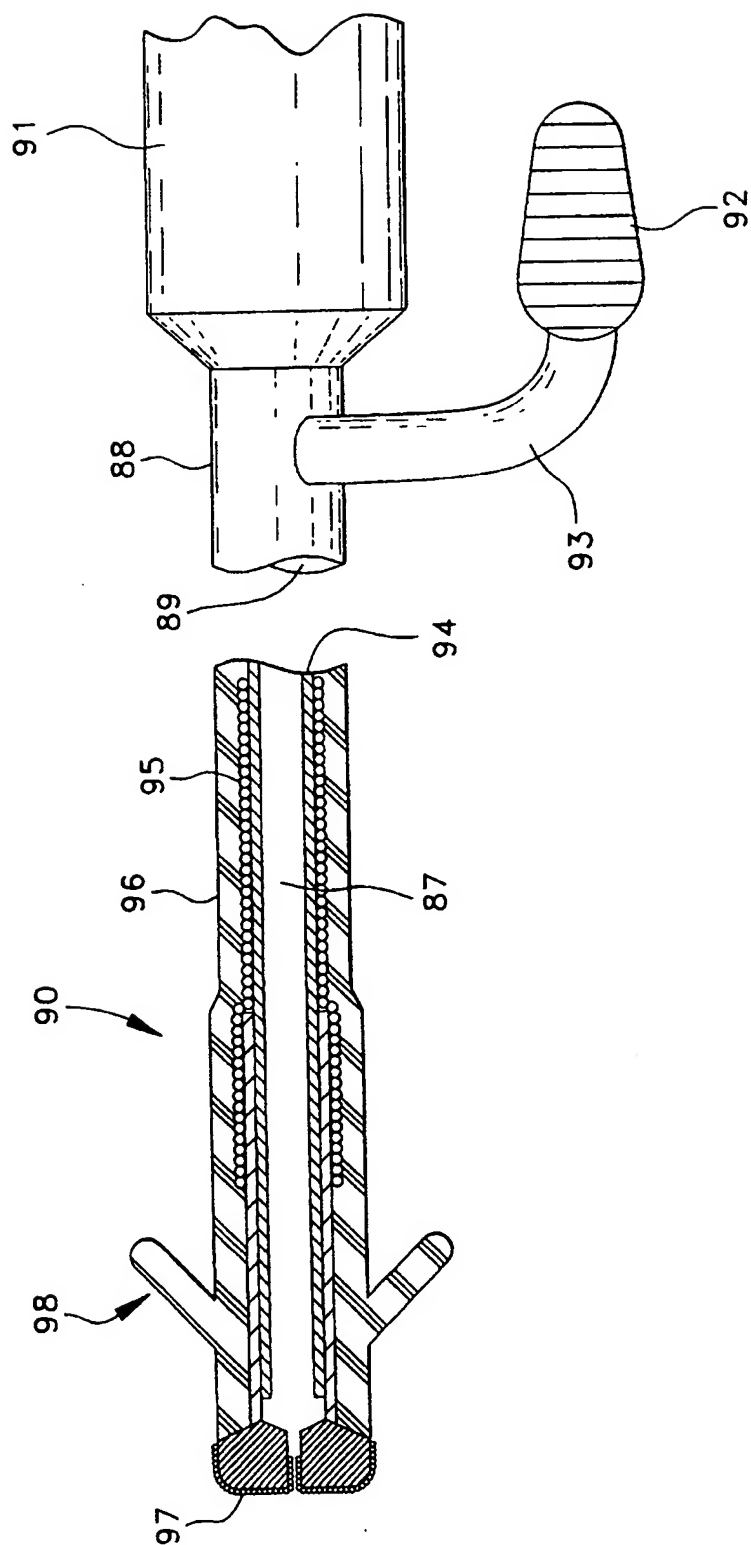


FIG. 5A

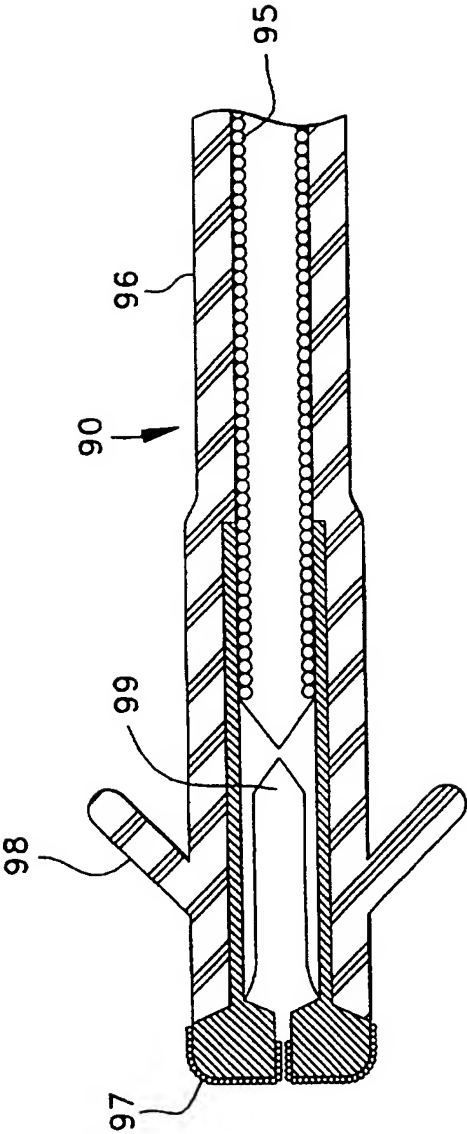


FIG. 5B

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US97/05556

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.  
US CL : 514/44; 536/23.1; 435/320.1; 607/120  
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 536/23.1; 435/320.1; 607/120

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
DIALOG: MEDLINE, BIOSIS, EMBASE, DERWENT; APS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,496,360 A (D.A.HOFFMAN) 05 March 1996, see abstract	1-35
Y	US 4,711,251 (K.B. STOKES) 08 December 1987, see abstract	1-35
Y	NABEL et al. Recombinant Gene Expression in Vivo Within Endothelial Cells of the Arterial Wall. Science. Vol. 244, pages 1342-1344, see entire document.	1-35
Y	GELLENS et al. Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage-dependent sodium channel. Proc. Natl. Acad. Sci. USA. January 1992, Vol. 89, pages 554-558, see entire document.	1-35

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means		
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search  
12 MAY 1997

Date of mailing of the international search report  
12 JUN 1997

Name and mailing address of the ISA/US  
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**INTERNATIONAL SEARCH REPORT**

International application No.  
PCT/US97/05556

**A. CLASSIFICATION OF SUBJECT MATTER:**  
IPC (6):

A01N 43/04; A61K 31/70; C07H 21/02, 21/04; C12N 15/00, 15/09, 15/63, 15/70, 15/74; A61N 1/04